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The tadpoles of *Scaphiophryne gottlebei* (Microhylidae: Scaphiophryninae) and *Mantella expectata* (Mantellidae: Mantellinae) from Isalo Massif, south-central Madagascar

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The tadpoles of the microhylid *Scaphiophryne gottlebei* and of the mantellid *Mantella expectata* from the Isalo Massif (south-central Madagascar) are described and compared with already known tadpoles belonging to the same genera. The tadpole of *S. gottlebei* is peculiar in having the oral apparatus with a horny beak surrounded by dermal papillae, and a spiracle in intermediate position, between ventral and lateral. Furthermore, it shows unique feeding habits and a peculiar associated behaviour. During the day it stays close to the bottom and often burrows half of the body into the sand with the tail obliquely upwards; in this position it ingests organic material from among the substrate particles. During night time the tadpole leaves the bottom and swims throughout the water column. Since this tadpole cannot be included in any of the currently known ecomorphological categories we create a new category for this species, the "*psammonektonic*" tadpole. *Mantella expectata* was often found in the same environments, although it appears to prefer more open habitats. In some cases, tadpoles of both species were found together, although *M. expectata* usually prefers small and temporary streams for reproduction. The mantella tadpoles were also found in quite open savannah areas. The tadpole morphology agrees in general with that of other mantellas, mainly of the *M. betsileo* group, and is of the generalized ranoid type.

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Introduction

Given the very high number of known species of amphibians in Madagascar (more than 220 according to Andreone & Luiselli, 2003, Andreone et al., 2005, and subsequent updates), it is not surprising that for most of them the tadpole morphology and general larval ecology are not yet known. Nevertheless, it is clear that the knowledge of the tadpoles is a crucial step in the assessment of conservation priorities, as it allows understanding the ecological requirements of the species in its whole and not only during the adult stage. Besides, the analysis of anuran larvae may help in the clarification of enigmatic phylogenetic positions that are hardly to be unveiled by studying only the adult characters. Finally, it is interesting to understand how the adaptation to peculiar and local ecological conditions is reflected in the larval ecology.

The conservation status of all the Malagasy amphibians was recently evaluated during the Global Amphibian Assessment (Andreone et al., 2005), which led to the identification of nine critically endangered species. Since the majority of these species (five of the genus *Mantella* and *Scaphiophryne gottlebei*) are (or have been until recently) important items in the pet trade, and therefore quite regularly kept in captivity (Andreone & Luiselli, 2003), it is surprising that the larval morphology is known for only one species, *Mantella aurantiaca* (Arnoult, 1965; Vences et al., 1999).

Thus, a series of surveys was recently carried out aimed at unveiling distribution and life history traits of these species. This was the case for *Scaphiophryne gottlebei* Busse & Böhme, 1992 and *Mantella expectata* Busse & Böhme, 1992, which are limited in distribution to the sandstone Isalo Massif, south-central Madagascar (Glaw & Vences, 1994). Incidentally both these species were described upon specimens imported for the trade, and until recently little was known about their life history traits (Busse & Böhme, 1992; Glaw & Vences, 1994). During an inventory in the Isalo Massif we had the opportunity to find the tadpoles of these species, for which we provide here descriptions.

Since the tadpole of the enigmatic genus *Scaphiophryne* was stated to be intermediate between the microhylid and ranid forms (Wassersug, 1984), we also took the opportunity to compare the *S. gottlebei*'s tadpole with those of other allied species. Its peculiar behaviour and habits led us to create a new ecomorphological category, discussed in detail below. Since both species turned out to be syntopic, we also provide information on their larval ecology.

Material and methods

Tadpoles were captured with a handnet during day and night inspections of the pools and other water bodies present in the wet canyons (and nearby areas) of the Isalo Massif, south-central Madagascar (Fianarantosa Province). They were maintained alive in small aquaria and fed with fish food. This allowed us to obtain a complete development series and to confirm their natural history traits via observations in a controlled environment. Preserved tadpoles are now housed in the Museo Regionale di Scienze Naturali, Torino (MRSN; see Appendix).

For *S. gottlebei*, the species identification was based on rearing the tadpoles until metamorphosis and on comparing the mitochondrial DNA of larvae (voucher specimen MRSN A2618) and of adults. For DNA study, we used standard extraction methods. A fragment of the mitochondrial 16S rRNA gene was amplified using the primers 16Sa-L and 16Sb-H of PALUMBI et al. (1991). Sequences were validated and aligned with the software Sequence Navigator (Applied Biosystems), and deposited in Genbank (accession numbers of newly obtained sequences from the tadpole: DQ078784). For *Mantella expectata*, the tadpoles were collected in a temporary pool and were reared until metamorphosis.

Tadpoles were photographed at different stages. A small number was euthanized by immersion in chlorobutanol, and successively fixed in 4 % formalin for morphological measurements. A few individuals were fixed and preserved in 90 % ethanol for genetical analyses. The remnant part was released at the capture site.

Terminology of measurements follows ALTIG & MCDIARMID (1999), whereas the labial tooth formula is given according to ALTIG (1970). Measurements were made at 0.01 mm under stereoscope, and are respectively based on 10 specimens at GOSNER's (1960) stages 25-44 for *S. gottlebei*, and on 20 specimens at stages 25-37 for *M. expectata*. Mean values and standard deviations are given in the descriptions (see tab. 1-2). We measured the following physicochemical parameters at two sites: pH (with Extech Extik PH100), conductivity (Extech Extick EC400) and oxygen (Extech D0407510).

RESULTS

TADPOLES' DESCRIPTIONS

Scaphiophryne gottlebei *Busse & Böhme, 1992 (fig. 1-3)*

The tadpole of this species exhibits a mosaic of different ecomorphological traits (MCDIARMID & ALTIG, 1999), and we propose for it a new ecomorphological category (see *Discussion*).

The body is stout and elliptical, flattened below, ovoid above. The snout is trapezoidal in dorsal view. The eyes are medium-sized, positioned dorso-laterally. The external nares are located dorsally, closer to eyes than to snout tip. They are visible and positioned in a slight light-coloured furrow. In tadpoles at advanced development stages (from 25 to 38), the narial apertures are apparently not open; they become clearly open at stage 41.

Tail fins are rather high. The dorsal fin is parallel to tail musculature, the ventral fin is higher than the dorsal, with its maximum height at about two-thirds of tail length. The dorsal fin originates at the tail-body junction and the ventral fin at the postero-ventral end of the body.

The spiracle is latero-ventral with a posterior opening. The inner wall of spiracle is absent. The vent tube is medial, ventrally directed, with a medial aperture.

Table 1. – Measurements (at 0.1 mm) of 10 tadpoles of *Scaphiophryne gottlebei*. GS, Gosner stage; *n*, number of specimens; TL, total length; TAL, tail length; BL, body length; BW, body width; E, eye diameter; IOD, inter-orbital distance; TMW, tail muscle width; TMH, tail muscle height; MTH, maximum tail height. Values are given as mean ± standard deviation. See the Appendix for locality references.

GS	*n*	TL	TAL	BL	BW	E	IOD	TMW	TMH	MTH
25		12.7 ± 1.9	25.3 ± 0.4	14.6 ± 5.1	4.8 ± 1.1	0.2 ± 0.1	2.5 ± 1.1	1.0 ± 0.0	1.7± 0.2	4.6 ± 1.6
26		14.3	26.1	11.8	4.8	0.2	3.0	1.2	2.0	4.5
27	1	21.0	37.0	15.5	18.0	0.3	5.0	1.2	3.0	7.0
33	1	23.5	40.8	17.3	10.0	0.4	5.7	2.0	3.5	8.0
34	1	25.0	41.5	16.5	8.5	0.3	6.0	1.3	3.1	8.2
38	1	29.1	48.2	19.1	11.0	1.6	8.0	2.6	5.0	–
41		25.7 ± 3.2	41.3 ± 3.2	16.0 ± 0.0	9.3 ± 0.4	0.4 ± 0.1	5.3 ± 0.4	1.8 ± 0.4	3.6 ± 0.4	7.0 ± 1.3
44	1	13.6	26.6	13.0	6.0	0.4	3.7	1.2	2.1	2.3

Table 2. – Measurements (at 0.1 mm) of 20 tadpoles of *Mantella expectata*. GS, Gosner stage; *n*, number of specimens; TL, total length; TAL, tail length; BL, body length; BW, body width; E, eye diameter; IOD, inter-orbital distance; TMW, tail muscle width; TMH, tail muscle height; MTH, maximum tail height. Values are given as mean ± standard deviation. See the Appendix for locality references.

GS	*n*	TL	TAL	BL	BW	E	IOD	TMW	TMH	MTH
25	2	15.6 ± 3.4	9.4 ± 2.1	6.2 ± 1.3	4.0±0.9	0.5± 0.1	1.3 ± 0.3	0.9 ± 0.3	1.1± 0.3	2.6 ± 0.6
26		19.7 ± 0.1	12.4 ± 1.8	7.3 ± 0.5	3.6 ± 0.3	0.6 ± 0.1	1.2 ± 0.2	0.9 ± 0.1	1.1 ± 0.3	2.4 ± 0.3
27		18.8 ± 4.9	11.0 ± 4.2	7.8 ± 0.7	3.8 ± 0.2	0.7 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	2.5 ± 0.2
28		20.0	12.0	8.0	4.0	0.7	1.5	0.9	1.1	1.3
35		25.1	14.8	10.3	5.7	0.9	1.5	1.7	1.7	4.0
37		28.6	17.8	10.8	6.2	1.1	1.8	1.8	2.6	4.6

The oral disc is roundish, not emarginated, with marginal and submarginal papillae tidily arranged all over the disc. The papillae are conical with rounded tips, sometimes with brownish pigment except at tips. Labial teeth are absent. Jaw sheaths are well developed. The inferior part of the lower jaw sheath is partially pigmented.

In life, the tadpoles are light greyish-brownish at night, shading to black during day, with sparse dark melanophores, denser in the dorsal and lateral posterior part of the body. A diamond-shaped translucent area is present between the eyes. Tail fins are transparent with a darker pigmented border on external edges, broader in the posterior end of ventral fin. Above the darker border, the tail is lightly scattered with dark spots. In preservative, the specimens become darker but maintain the natural pattern. Tadpoles near metamorphosis begin to acquire the adult pattern. Tadpoles in formalin kept the overall natural colour pattern, whereas tadpole in ethanol showed a general shrinkage and loss of colour. Metamorphosing toadlets are 10-15 mm long, with an overall coloration (white, red and black) similar to that of the adults, although apparently less contrasted.

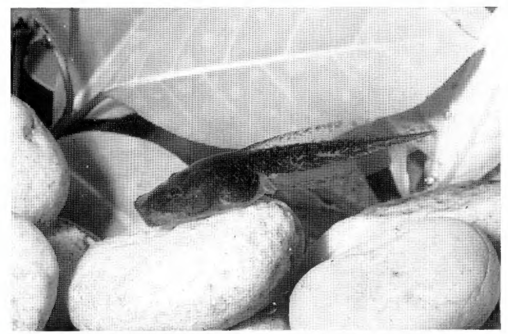


Fig. 1. – Lateral view of a tadpole of *Scaphiophryne gottlebei*. MRSN A4961, Gosner stage 38 (total length 29.1 mm), from Zahavola, Isalo Massif.

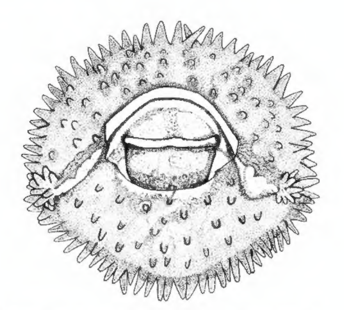


Fig. 2. – Oral disc of *Scaphiophryne gottlebei* (based upon MRSN A2618) at Gosner stage 38.

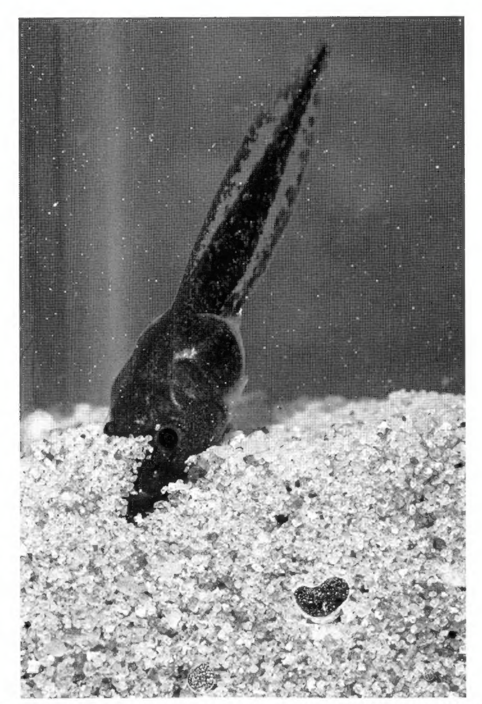


Fig. 3. – Tadpole of *Scaphiophryne gottlebei* half-buried in the sand, a typical position assumed during the day.

Observations in captivity and in nature confirmed that during the day the tadpoles stay close to the bottom and often burrow within the bottom's substrate with half the body embedded in the sand and mud and with the tail projecting obliquely upwards at an angle of about 30-45°. In this position, the tadpoles ingest particles of the substratum. At night they leave the bottom and swim throughout the water column, often reaching the surface where they ingest air.

Mantella expectata *Busse & Böhme, 1992 (fig. 4-5)*

These tadpoles are of the benthic type (McDIARMID & ALTIG, 1999).

The body is elliptical in lateral view and ovoid in dorsal view. The snout is dorsally rounded, whereas in lateral view it slopes gently to the oral region and then turns strongly. External nares are located dorso-laterally, almost half way from eyes to snout tip. The eyes are small and directed dorsally.

Tail fins are low and of about equal height, the dorsal fin being lower than the ventral at the level of the vent tube. The dorsal fin originates near the tail-body junction, and the ventral fin at the posterior ventral end of the body. The maximum tail height is at the middle of the tail. The tail tip is rounded with the tail muscle almost reaching the end of the tail.

The spiracle is sinistral with a mid-lateral opening directed posteriorly. The inner wall of the spiracle is present and free from the body. The vent tube is parallel with the ventral margin of the fin, tubular in shape and displaced dextrally, with a medial aperture.

The oral disc is antero-ventral, elliptical, emarginated, with a uniserial row of marginal papillae in the lower labium and on the lateral side of the upper labium. Few submarginal papillae are present in the lateral portions of the upper labium. The papillae are conical, with rounded tips, unpigmented and translucent. The labial tooth row formula is 5(2-5)/3(1). The upper jaw sheath is flat on its large medial part with a median concavity, the lower jaw sheath is V-shaped; both are finely serrated and entirely pigmented in black.

In life, these tadpoles are uniformly brownish and speckled with sparse melanophores, denser in the dorsal and lateral posterior part of the body. Tail fins are mainly transparent, slightly scattered with dark spots, especially the dorsal fin. In preservative, the specimens maintained the natural colour pattern.

The morphology of *Mantella expectata* tadpoles is similar to that of other mantellas of the *M. betsileo* group, being of a generalized ranoid type. Tadpoles close to metamorphosis begin to acquire the coloration typical of most of *Mantella* species: the back is brownish-yellowish, and the flanks blackish. At metamorphosis the froglets measure about 10 mm.

HABITAT DESCRIPTION

The sandstone Isalo Massif is located within the Central Ecoregion (ANONYMOUS, 2003). At the closest town, Ranohira, the mean monthly temperature is 25.1° C, with an absolute minimum of 3.4° C (June); precipitation is concentrated in the rainy season from late October to February (ANONYMOUS, 1999).

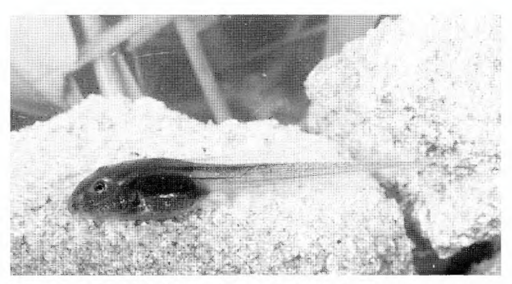


Fig. 4. – Lateral view of a tadpole of *Mantella expectata*, MRSN A3435, Gosner stage 37 (total length 28.5 mm) from Zahavola 1, Isalo Massif.

Fig. 5. – Oral disc of *Mantella expectata* (MRSN A3435) at Gosner stage 37.

To provide indications about the ecological preferences in adults and tadpoles of *S. gottlebei* and *M. expectata*, it is necessary to give an overall description of the Isalo Massif in terms of habitat availability.

The three main habitat types recognised within the Isalo Massif are related to the peculiar topography: (1) the savannahs, (2) open valleys, and (3) narrow canyons.

(1) The savannahs are subject to repeated fires and are covered with extensive meadows with scattered trees and isolated forest parcels. The night-day temperature difference is high, and the humidity is usually very low. Aquatic habitats are represented by temporary pools (often used for cattle), streams and rivers. The temporary rivers are filled by seasonal rains, and are dry for most of the year. A few permanent or semi-permanent rivers are present and may be accompanied by gallery forests. At these habitats we found species which breed in temporary waters (e.g., *Boophis occidentalis*, *Laliostoma labrosum*, *Ptychadena mascareniensis*, *Scaphiophryne brevis* and *Dyscophus insularis*).

(2) The open valleys are usually crossed by permanent or semi-permanent torrents with quite wide water beds, cascades and pools, and gallery forests of various sizes. We found frog species that usually need permanent water to breed, such as *Mantidactylus* cf. *femoralis*, *Boophis goudoti* and also *Boophis occidentalis*.

(3) The rocky and montane part is crossed by canyons of various lengths, widths and depths, and with a variable water presence. Some canyons are very narrow with a sandy bed delimited by vertical rock walls. The habitat is dark and sometimes quite similar to a cave, with a rather low and constant temperature (19-22°C) and high humidity (about 100 %). Within these close canyons, vegetation is absent (due to scarcity of light) or limited to a few isolated trees. Typical species of this habitat are *Scaphiophryne gottlebei*, *Mantidactylus corvus* and *Mantella expectata*.

The canyons can be ideally divided in four tracts, although not all of them are always present. (a) savannah tract, with absent to low walls (0.5-1.5 m high), grass vegetation, sandstone soil substratum with cobbles, small pools (0.1-0.2 m deep) with little or no water, exposed to sunlight and subject to strong evaporation; (b) initial tract with medium-high walls (1.5-5.0 m), if present high arboreal or shrub-like vegetation in the floodwater bed, thin sandstone substratum with cobbles or isolated stones, and deep water filled pools (0.5-1.5 m), sometimes exposed to sunlight, (c) gully tract, with high to very high vertical walls (5.0 m and more), no vegetation, thin sandstone soil or rocky substratum, very deep water-filled pools (1.5 m and more), generally no sunlight exposure (in some cases this tract may have a cave-like aspect), (d) terminal tract, with high to very high vertical or concave walls directly in contact with the watershed, possibly temporary waterfalls, absent or scarce flood-water bed vegetation, water-filled pools of different depths, temporarily exposed to sunlight.

Adults of *S. gottlebei* were usually found within the canyons, where they burrow in the sandy substrate or hide in cavities in the walls. In rare cases we found them outside the canyons. As a consequence, the tadpoles were usually found in temporary pools excavated by running water in the rocks within the narrow canyons, gully and terminal tracts (fig. 6). In some cases, especially after heavy rainfalls, tadpoles were found in the initial tract. In December 2004, we found tadpoles that had likely hatched at the beginning of October after



Fig. 6. – Habitat of *Scaphiophryne gottlebei* at Malaso, Isalo Massif. Gully tract of the canyon, with deep and semi-permanent water pools.

more than two months, they were still without hind legs. For this reason, we suspect that metamorphosis in this species takes 2-3 months, according to the local climatic conditions.

Adult individuals of *M. expectata* were found in open areas along the small streams quite exposed to the sun. We usually did not observe the mantellas within the real canyons, although in some occasions they were seen at the initial tract. The tadpoles were found in the small pools in open areas, only rarely within the canyons. Tadpoles of *M. expectata* were generally found in the savannah and initial tract of canyons, quite exposed to the sunlight, although in some cases they could be present in the other tracts (fig. 7). *M. expectata* breeds and completes its larval development in about 1-2 months.

We also measured the chemical water parameters at two of the studied sites: (1) "Zahavola 2" (presence of *S. gottlebei* tadpoles), water temperature 24°C, pH 5.23, conductivity 10.04 µS/cm, O_2 8.8 mg/l; (2) "Zahavola 3" (presence of *M. expectata* tadpoles), water temperature 26.6°C, pH 7.25, conductivity 8.07 µS /cm, O_2 2.7 mg/l.

DISCUSSION

The discovery and description of the tadpole of *Scaphiophryne gottlebei* allows us to make some preliminary comparisons with the general morphology of tadpoles of other species belonging to the genus *Scaphiophryne*. At present only the tadpoles of *S. calcarata* and of the recently described *S. menabensis* are sufficiently known (BLOMMERS-SCHLÖSSER, 1975; BLOMMERS-SCHLÖSSER & BLANC, 1991; GLOS et al., 2005).

Concerning *S. calcarata*, the line drawing and the written description of the tadpoles suggested that they were nektonic. The text also indicated that their beak was not keratinised. As stressed in WASSERSUG's (1984) study of the internal anatomy, this statement was wrong, since these mouthparts are keratinised. Apart from this, the description of this tadpole does not differ much from what we report for *S. gottlebei*. Both species have a terminal mouth surrounded by dermal papillae. Possible differences concern the lack of the extended flap on the lower lip and the narial position, which appears nearer to the tip of the snout in *S. calcarata* and nearer to the eye in *S. gottlebei*.

The tadpole of *Scaphiophryne gottlebei* differs from that of *S. menabensis* by body shape, by narial distances (nearer to the eye vs. same distance to snout tip and to eye). Similarities are shared in the morphology of the oral disc with unpigmented jaw sheaths and marginal conical papillae, and on the displacement of the spiracle (GLOS et al., 2005).

The tadpoles of *Scaphiophryne gottlebei* also show unique feeding habits and an associated particular swimming behaviour. During the day they usually stay close to the bottom and burrow within the substrate, propelled by intermittent movements of tail and body, with half the body dug into the sand and with the tail obliquely upwards (at an angle of 35-40°). In this half-buried position they ingest particles from the mud and sand substratum. In fact, in all collected tadpoles the intestine was completely filled with detritus. During night time the tadpoles leave the bottom and swim throughout the water column while apparently filtering suspended particles. As far as known the only other tadpoles that show somewhat similar habits belong to the microhylid *Otophryne robusta*. This tadpole is a passive filter-feeder in a



Fig. 7. – Habitat of *Mantella expectata* at Lola, Isalo Massif. It is represented by a temporary stream at the beginning of the initial tract of the typical montane canyons.

full fossorial habitat with related unique morphological features (WASSERSUG & PYBURN, 1987)

The odd feeding habits make it hard to place the *S. gottlebei* tadpole in the ecomorphological categories of MCDIARMID & ALTIG (1999). If forced into this classification, it should be considered as intermediate between "suspension feeder type 2" and "suspension rasper" and between "benthic" and "psammonic". For this reason we coined the name "*psammonektonic*" for a new ecomorphological category. This category describes a tadpole with keratinised mouthparts and papillae, ventro-lateral spiracle, dorso-lateral eyes, feeding partially by filtering suspended particles within the water column and by direct ingestion of substratum through active burrowing, and active day and night.

Four other *Scaphiophryne* species (*S. brevis*, *S* sp. from Andringitra [formerly attributed to *S madagascariensis*], *S. madagascariensis* from Ankaratra, and *S marmorata* from Andasibe area) were cursorily described by GLAW & VENCES (1994), VENCES et al. (2002) and BIGGI (2002), but none of these data allows any detailed comparison. Anyhow, from the observations and photographs in these publications, we presume that the tadpoles of these species are similar to that of *S gottlebei* in having: (1) a ventro-lateral spiracle, (2) keratinized jaw sheaths, (3) an absence of teeth, (4) dorso lateral eyes, (5) a general robust body shape, and (6) suspension and/or macrophagous feeding habits. Furthermore, these tadpoles are also transitional between benthic and nektonic morphotypes and feed on small particles. The general morphological similarity is also confirmed by the photograph of *S madagascariensis* from Ankaratra (VENCES et al , 2002), that shows a tadpole very similar to that of *S gottlebei* herein described.

Of the above mentioned characters, the presence in *S. madagascariensis* of keratinised jaw sheaths, described by GLAW & VENCES (1994), has been recently confirmed by HAAS (2003) Maybe, as observed also in the tadpole description of *S calcarata*, the presence of unpigmented but keratinised jaw sheaths lead previous authors to mistake as they assumed that keratinized tissue has to be black

A more detailed comparative analysis of the *Scaphiophryne* tadpoles is much needed because the scaphiophrynines have so far been alternatively included in the Ranidae, Microhylidae or Hyperoliidae families (WASSERSUG, 1984) or even in a separate family (DUBOIS, 1992). The type 2 larva of ORTON (1953, 1957) was generally considered diagnostic of the Microhylidae, but indeed larvae of scaphiophrynines and many other microhylids remain unknown. As shown by BLOMMERS-SCHLÖSSER's and WASSERSUG's works, now confirmed by the description of the *S gottlebei* tadpole, the tadpole groups after ORTON often appear inadequate to provide clear phylogenetic information. Furthermore, the inclusion the genus *Paradoxophyla* within the Scaphiophryninae should be re-investigated, as this genus has a specialised filtering tadpole (ANDREONE et al , 2006) So far, the information available does not provide an unequivocal indication.

Finally, tadpoles of the genus *Mantella* are less crucial in determining phylogenetic allocation because they belong to the typical ranoid morph Moreover, the genus *Mantella* appears very homogeneous in terms of morphology and ecology The only detailed data were reported for *M aurantiaca* by ARNOULT (1965) and later summarised by BLOMMERS-SCHLÖSSER & BLANC (1991) Indeed, both species share a labial tooth row formula of 5(2-5)/3(1) and have an emarginated oral disc with papillae on the lower labium Papillae in *M*

expectata are displaced in a uniserial row whereas in *M. aurantiaca* they are apparently biserial. In contrast, *M. laevigata* differs in having a reduced labial formula of 3(2-3)/3 or 4(2-3)/3(1-3) and a stronger and more notched horny beak (GLAW & VENCES, 1994). Further comparisons with other species are not possible because of lack of information.

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APPENDIX

LIST OF EXAMINED SPECIMENS

All the collecting sites are within Ranohira Fivondrononana, Fianarantsoa Faritany, Madagascar. An asterisk (*) indicates ethanol fixed specimens.

Scaphiophryne gottlebei Busse & Böhme, 1992: MRSN A2618 (*n* = 1) and A2619* (*n* = 1), Isalo Massif, Parc National de l'Isalo, Vallee du Petit Nazareth, 22°32.91'S, 45°21.72'E, 890 m, leg V Mercurio, 2.II.2004; MRSN A4961 (*n* = 3), Isalo Massif, Parc National de l'Isalo, Marojana River, 22°27 43'S, 45°22 40'E, 867 m, leg. V Mercurio, 15 XI 2004; MRSN A4962 (*n* = 6), Isalo Massif, Zahavola 2, 22°37.38'S, 45°21 52'N, 825 m, leg. F. Andreone, F. Mattioli & V. Mercurio, 20.XI.2004

Mantella expectata Busse & Böhme, 1992. MRSN A3432 (*n* = 22) and MRSN A3433 (*n* = 23), Isalo Massif, Andranomena, 45°18 86'E, 22°45 71'S, 786 m, leg F Andreone, V Mercurio & J. E. Randrianirina, 28 I 2004, MRSN A3434, (*n* = 2), Isalo Massif, Parc National de l'Isalo, Zahavola 3, 45°21 48'E, 22°37 51'S, 835 m, leg V Mercurio, 2 II 2004; MRSN A3435 (*n* = 2), Isalo Massif, Parc National de l'Isalo, Andohasahenina, 45°17 28'E, 22°49 79'S, 630-680 m, leg. F Andreone, G. Aprea, V Mercurio & J E Randrianirina, 15 I.2004.

Alytes, 2006, **23** (3-4): 96-102.

Description of the tadpole of the Malagasy treefrog *Boophis andohahela*

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We describe the larval stages of the Malagasy treefrog *Boophis andohahela*, based on specimens identified by their DNA sequences. The tadpoles were collected in a stream pool under a waterfall and were dwelling on submerged rocks. They show a rather distinctly flattened and convex body shape. Their oral disk structure and labial tooth row formula (2:4+4/1+1:2) is similar to those of other representatives of the *Boophis luteus* species group.

INTRODUCTION

The genus *Boophis* Tschudi, 1838 contains a radiation of treefrogs which belongs to the endemic family Mantellidae from Madagascar and the Comoro island of Mayotte (VENCES et al., 2003). The genus currently contains about 48 species (GLAW & VENCES, 2003), but new taxa are continuously being discovered, and many species have been already identified and await formal description (VALLAN et al., 2003). Frogs of this genus are arboreal, with typical treefrog habitus, enlarged finger discs, broad and anteriorly rounded head, large eyes and no dorsolateral ridge (GLAW et al., 2001). According to BLOMMERS-SCHLÖSSER & BLANC (1991) and GLAW & VENCES (1994), seven phenetic species groups are distinguished in the genus.

Within *Boophis*, two major clusters can be distinguished depending on the site of reproduction: the pond breeders of the *Boophis tephraeomystax* group appear to be characterized by ancestral states of several characters (VENCES et al., 2002) but they were grouped as a homophyletic group in a more recent analysis (VENCES et al., 2003). It is clear, however, that

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the species-rich assemblage of brook-breeders is a homophyletic, probably monophyletic group (RICHARDS et al., 2000; VENCES et al., 2002, 2003)

One of the species assemblages in this lotic lineage is the *Boophis luteus* group that contains a number of morphologically extremely similar, medium-sized green-coloured treefrogs. The number of species in this group has climbed up from one (BLOMMERS-SCHLOSSER & BLANC, 1991) to 12 (GLAW & VENCES, 2002). Larval stages are known for only three of these, *Boophis luteus* (Boulenger, 1882), *Boophis ankaratra* Andreone, 1993 and *Boophis jaegeri* Glaw & Vences, 1992 (BLOMMERS-SCHLOSSER, 1979; GLAW & VENCES, 1994) We here describe the tadpole of one further species of the *B. luteus* group, *Boophis andohahela* Andreone, Nincheri & Piazza, 1995.

MATERIAL AND METHODS

Specimens were collected in January 2003 in Ranomafana National Park, Fianarantsoa Province, southeastern Madagascar, from a brook in the rainforest The habitat was a pool underneath a waterfall (ca. 847 m above sea level, 21°15.77'S, 47°24.78'E), which dropped down about five meters along rocks. The pool was very deep (more than 2 metres) and had a diameter of at least seven metres Specimens were attached to the submerged rocks in the pool and were found on rocks in quiet water areas as well as on rocks positioned in strong current. Collected specimens were anesthetized and killed in a solution of highly concentrated chlorobutanol The dead tadpoles were assigned to morphotype categories using a stereomicroscope From one specimen of each of these categories a piece of tail was taken as a DNA tissue sample. Subsequently all tadpoles were preserved in 4 % buffered formalin. Adult and larval voucher specimens were deposited in the herpetological collections of the Université d'Antananarivo, Département de Biologie Animale (UADBA), Zoologische Staatssammlung Munchen (ZSM) and the Zoological Museum Amsterdam (ZMA).

Species identification was based on DNA sequences. We amplified a fragment of about 500 bp of the mitochondrial 16S rRNA gene of each tadpole sample, using primers and protocols described in THOMAS et al. (2005), and compared it with homologous sequences of adult specimens. DNA sequences were deposited in Genbank (accession numbers AY863216-AY863217 for the two tadpole DNA vouchers, and AY848447-AY848448 and AY848456 for three comparative adult specimens)

Drawings and descriptions are based on the DNA voucher, and other representative specimens of the same series were used to supplement structures missing because of tissue sampling In order to assess morphological variability, measurements were taken from six specimens of the series using dial calipers; values were taken to the nearest 0.1 mm All tadpoles were staged according to GOSNER (1960) Terminology is based on ALTIG & MCDIARMID (1999) with some modifications. Body length is estimated by measuring the distance from the tip of the snout to the body terminus, which is the junction of the posterior body wall with the tail axis (ALTIG & MCDIARMID, 1999). Tail length is defined as the distance from the body terminus to the absolute tip of the tail (ALTIG & MCDIARMID, 1999) Total length is the sum of body length and tail length Body width is measured at the widest point

of the "head" right behind the eyes, not in the intestinal part. Eye diameter is the maximum width of the orbit. Interorbital distance is measured between the centres of the pupils; internarial distance is measured between the centres of the nares. The distance between tip of snout and naris is taken to the centre of the naris. Distance between naris and eye is measured from the centre of naris to the anterior edge of the eye. Distance between tip of snout and spiraculum is also taken up to the centre of the spiracular aperture. Tail muscle height is first measured vertically from the junction of the body wall with the ventral margin of the tail muscle and secondly measured at midtail. Tail height including fins and caudal musculature is taken at its maximal vertical extent. Dorsal fin origin is defined relatively to the tail body junction. The formula of labial tooth rows follows Dubois (1995). The mouthparts include upper tooth rows (UTR) and lower tooth rows (LTR)

Results

Boophis andohahela was described from Andohahela National Park in south-eastern Madagascar (Andreone et al., 1995). Our surveys of south-eastern rainforests yielded, in 2003 and 2004, several specimens that agreed with this species in general morphology and coloration: (1) at Ambatolahy forest next to Ranomafana National Park, 21°14.632'S, 47°25.573'E, 915 m a.s.l. (specimens ZMA 20017-20018 and 20304, collected in February 2004); (2) close to the first locality, between Vohiparara and the entrance of Ranomafana National Park, no coordinates taken (specimens ZSM 665.2003, collected on 17 January 2003); (3) at Vevembe forest, close to Vondrozo, 22°47.686'S, 47°11.228'E, 581 m a.s.l. (specimens ZMA 20019 and 20125-20126, and UADBA 24292, collected on 10 February 2004). Specimens from Vevembe were observed calling, their advertisement calls fully corresponding to those of topotypical specimens as described by Andreone et al. (1995). DNA from three of these adult specimens was sequenced, the two sequences from the Ranomafana region (specimens ZMA 20018 and ZSM 665.2003) resulting fully identical, the one from Vevembe (ZMA 20125) having 6 substitutions compared to those from Ranomafana (1.2 % pairwise sequence divergence)

Two tadpole series from Ranomafana with the field numbers FG/MV 2002.1802 (catalogued as ZSM 667.2004) and FG/MV 2002.1803 (catalogued ZSM 668.2004) had sequences fully identical with the adult sequences from Ranomafana, and their sequences strongly differed from all other frog species studied in this region. In terms of DNA barcoding we therefore consider these tadpoles to be reliably identified. We based the following description on a subset of the specimens from one of these series (ZSM 667.2004). Specimens from the second series agreed in general morphological features.

Larvae of *B. andohahela* are exotrophic and benthic tadpoles of Orton's (1953) type IV. The coloration shows irregular pattern of dark areas on a light ground. The intestinal spiral is clearly visible through the abdominal wall. In life, most of the observed specimens showed a yellow coloration on the tail: the fins were almost without pigmentation, just a yellow glimmer was visible

We selected the DNA voucher of the series ZSM 668.2004 and five additional tadpoles of this same series, of representative size and stage, and in good state of preservation, for the

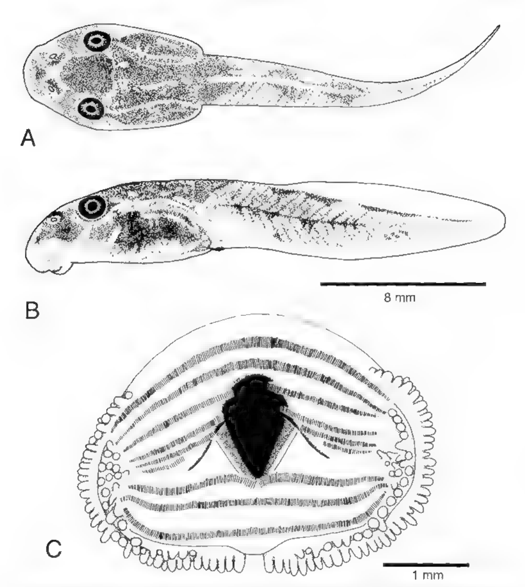


Fig 1 Drawings of a tadpole of *Boophis andohahela* from the series ZSM 667 2004 On top (A) the specimen is shown in dorsal view with its relatively large eyes, in lateral view (B) the very low body shape is visible, the oral apparatus (C) shows the dense row of marginal papillae with its large medial gap in the upper labium and its small gap in the lower labium

description. The DNA voucher specimen had a part of the tail removed for DNA extraction. All specimens were in stage 25. Detailed morphometric data of the specimens are given in tab. 1. The larvae of *B andohahela* have a total length of 21 84 ± 0.76 mm (mean ± standard deviation) They show an oval to more of less rhombic body shape in dorsal view (fig 1A) and the body width is about 58 % of body length The snout is flatly rounded, and the upper

Table 1. – Morphometric measurements (mm) of six tadpole specimens of *Boophis andohahela* (series ZSM 667/2004, all in stage 25) collected in Ranomafana National Park.

Character	*n*	Mean	Standard deviation	Minimum	Maximum
Body length	6	8.03	0.66	7.3	8.9
Tail length	5	13.98	0.58	13.5	14.9
Total length	5	21.84	0.76	20.8	22.6
Body width	6	4.66	0.52	4.3	5.7
Eye diameter	6	1.20	0.11	1.1	1.4
Interorbital distance	6	3.02	0.19	2.9	3.4
Internarial distance	6	1.37	0.05	1.3	1.4
Distance snout-naris	6	1.25	0.12	1.0	1.3
Distance naris-eye	6	1.78	0.12	1.6	1.9
Distance snout-spiraculum	6	4.82	0.38	4.3	5.4
Tail muscle height 1	6	2.60	0.09	2.5	2.7
Tail muscle height 2	6	2.05	0.08	2.0	2.2
Fin height	6	3.30	0.28	2.8	3.6

mouthpart is anterior. The eyes are relatively large (diameter about 15 % of body length). They are positioned dorsally and directed dorsolaterally. In ventral view the eyes are not visible. The internarial distance is about 45 % of the interorbital distance. The rounded naris is moderate in size, directed dorsally and positioned closer to the snout than to the eyes. In lateral view (fig. 1B), the body shape is very depressed and in some specimens shows an extreme concave shape ventrally. The snout is rounded. The spiracle is sinistral and ¾th of the tube are attached to the body wall, it is positioned laterally (closer to venter than to dorsum) and oriented posterodorsally. The spiracular opening is oval and situated slightly below the level of the apex of myotomes of tail musculature. The tail musculature is strong, of almost uniform height until the midtail, in the distal half of the tail the musculature is gradually tapering and almost reaches the tail tip. The fins are moderate. The dorsal fin originates near the dorsal tail body junction, but really expands just after one fourth of the tail length. Like the ventral fin, the dorsal fin has a concave shape. The point of maximum fin height is located in the third fourth of the tail. The anal tube is short, tubular and medial with a lateral displacement to the right, the opening is directed posterolaterally.

The oral apparatus (fig. 1C) is generalized. It is positioned ventrally and there is no lateral emargination present. The upper labium shows a large medial papillae gap. The rest of the oral disc is bordered by a dense row of marginal papillae, except a small part in the middle of the lower labium. Submarginal papillae are present in the lateral parts and cover almost the whole lower labium, just a small area in the middle being free of submarginal papillae. The labial tooth row formula is 2:4+4/1+1:2. In the upper labium, the tooth rows become continuously shorter from UTR_2 to UTR_6. UTR_3 is the first row that touches the beak. LTR_1 has a short medial gap. The jaw sheaths are slightly serrated, the coloration is white with black pigmentation. On the upper labium the beak has a wide opened reversed U-shape, whereas the lower beak is a compact element with a slight V-shaped grooving.

DISCUSSION

DNA barcoding has proven to be a valuable tool to assign larval stages to adult species, especially in cases were rearing would be very time-consuming (HEBERT et al., 2004; THOMAS et al., 2005). In the case study reported here, we have even used this method first to assess the conspecificity of adult specimens from several localities, and in a second step to verify tadpole identification. In *Boophis andohahela*, as in other species of the *B. luteus* group, the original green colour quickly fades to yellow and later to white, with the slight species-specific chromatic characters totally vanishing. Even living frogs have few diagnostic characters, and the most distinct one (light dorsolateral lines on the anterior part of the body) can also be found in other species. Hence, the only adult specimens in our collection that could be reliably identified using traditional methods were those from Vevembe, because here we could collect them while emitting their diagnostic advertisement calls (described by ANDREONE et al., 1995). These differ clearly from those of all other representatives of the *B. luteus* group, except *B. jaegeri* (see GLAW & VENCES, 2002) which strongly differs genetically. Adult specimens collected at Ranomafana were assigned to *B. andohahela* because of agreement in live coloration and low genetic differences to a specimen from Vevembe. In turn, tadpoles from Ranomafana were identical in their DNA sequence to adults from this region. Altogether five DNA sequences of *B. andohahela* (two tadpoles and three adults) were available, and the differences among these were much lower than to all other species of *Boophis*, confirming the validity of molecular taxonomy to identify larval stages of tropical anurans.

According to BLOMMERS-SCHLÖSSER (1979) and GLAW & VENCES (1994), the tadpoles assigned to *B. luteus* and *B. jaegeri* are characterized by the following morphologies: labial tooth formula 1.5+5/3 or 1:4+4/3 with a large number of papillae, gap in papillae on the upper labium and median gap on the lower labium; body not conspicuously flattened in *B. luteus*, slightly flattened in *B. jaegeri*. Hence, the general oral morphology of *B. andohahela* agrees relatively well with its close relatives. Its rather flattened, almost concave ventral body shape might be an adaptation to adhesion to submerged rocks in strong currents and reminds tadpoles of *B. ankaratra* (as briefly described in GLAW & VENCES, 1994) and of representatives of other species groups, *Boophis majori* (Boulenger, 1896) (*Boophis majori* group), *Boophis erythrodactylus* (Guibe, 1953) and *Boophis mandraka* Blommers-Schlosser, 1979 (*Boophis rappiodes* group). This indicates that several characters of the tadpole morphology in *Boophis* have undergone extensive parallel evolution in similar habitats. Deciphering the pathways and ecological correlates of the recurrent adaptations to more or less extreme lotic conditions must await a better knowledge on the phylogeny of these frogs, and the descriptions of the larval stages of more species.

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Description of advertisement calls of six species of the genus *Chaparana* (Ranidae) from Nepal and India

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The advertisement calls of six species of the genus *Chaparana* (subgenus *Paa*) are described in details, those of two of them (*Chaparana minica* and *Chaparana vicina*) for the first time. For each species various temporal and frequency parameters are given. Each call is illustrated by an oscillogram, a spectrogram and a spectrum. The general characteristics of these peculiar calls are discussed as adaptations to a noisy torrent environment. The calls and modes of life of these frogs are consistent with their taxonomy based on morphological and molecular characters.

INTRODUCTION

This paper is devoted to the description of the advertisement calls of six species of the subgenus *Paa* of the genus *Chaparana* Bourret, 1939, as redefined by OHLER & DUBOIS (2006). This genus of the ranid tribe Paini (DUBOIS, 1992; OHLER & DUBOIS, 2006) includes 26 species distributed in South and Southeast Asia (Pakistan, India, Nepal, western China through Myanmar, Thailand, Laos, Cambodia and Vietnam) (DUBOIS, 1976; FROST, 1985; FEI, 1999). The species of the genus *Chaparana* are torrent-living species. The males of these species usually call at night from the bed of the torrent, very often hidden under stones and rocks or below the bank, more rarely sitting in the water in more open places of the torrent bed (DUBOIS, 1976, 1977*b*). They are distributed along the torrent which avoids interactions between them (DUBOIS, 1977*b*). The loud and continual background noise of the running water as well as their calling sites triggered the frogs to develop an advertisement call adapted to this environment, a type of call seldom encountered in the other species of Ranidae, except for those living in the same kind of habitats, such as *Amolops* (DUBOIS, 1977*a*). The calls of some of the species described hereafter have already been briefly described by DUBOIS (1977*b*). We decided to redescribe them to provide parameters which were not measured at that time and to give standard descriptions of calls which can be used for comparison in future studies.

The calls of six species of *Chaparana (Paa)* were recorded during field work in Nepal and India by the second author in 1972, 1973 and 1977 (tab. 1). However, the distributions of these species are larger: *Chaparana (Paa) rostandi* lives in Nepal only, *Chaparana (Paa) vicina* occurs in India and Pakistan, *Chaparana (Paa) minica* in India and Nepal, and *Chaparana (Paa) blanfordii*, *Chaparana (Paa) liebigii* and *Chaparana (Paa) polunini* are found in Nepal and China (Dubois, 1976, 1980, 2000, Frost, 1985). They are all mountain species which occur mostly at high altitudes.

Material and methods

Recordings (tab. 1) were made using either a Uher Report 4000 or a Sony TCDM-5 tape recorders, and Scotch magnetic 215 and TDK SA-X90 tapes. Oscillograms, spectrograms and spectrums were prepared with the software tool Canary 1.2 from the Cornell Laboratory of Ornithology (Charif et al., 1995). The sampling rate used to convert the signals to digital format was 22 254 Hz with 16-bit precision. A filter bandwidths of 349.70 Hz and frame length of 512 points were used for both spectrogram and spectrum analyses.

The following measurements were taken from the oscillogram duration of calls (dc), duration of notes (dn) and intervals between notes (din), number of notes per call (nn) and note rate (number of notes per second, nns). Frequency measurements were made from the spectrum of a few notes within the signals. The frequency values given (in text and tab. 2) are the means of the values of the same frequency band of those notes. The visible frequency bands are noted f1b to f5b (when possible) from the fundamental frequency to the highest harmonic

The recording of the call of *C. vicina* was interfered by a significant background noise. So we used the software Signalyse 3.10 which proves to be more efficient to filter signals: frequencies below 300 Hz and above 500 Hz were cut off, oscillograms and spectrogram were built with Canary 1.2. The call of *C. minica* was recorded with deficient batteries in the tape recorder. So one can hear an important speed difference when the tape is played with a Uher in good condition. The tape was re-recorded using the human voice on the tape as an indication for the adequate speed (original speed 17.6 cm/s instead of 19.0 cm/s, that is to say 7.5 % of the normal speed). Therefore there is a probable (slight) error on the measurements of the parameters for time and frequency. The latter should be considered with caution and rounded off (the margin of error could not be estimated) However it seemed useful to publish a description of this call as data concerning this species are rare.

All the specimens are deposited in the collection of the Muséum National d'Histoire Naturelle of Paris (MNHN)

Except for *C. vicina*, no males whose calls are studied here could be caught, so it is impossible to give a voucher number for each animal Snout-vent lengths were estimated by averaging the snout-vent lengths of males caught in the same population as the singer (Dubois, pers. obs.). The MNHN collection numbers of the individuals taken into consideration for calculation of the means are the following: (1) *C. blanfordii*, 1975 1056-1058, (2) *C. liebigii* 1975 1093, 1975 1097-1098, 1975 1102, 1975.1105-1107, 1975.1109-1112, (3)

Table 1. List of the species studied with information on the place and date of recording. Air T°: air temperature; Water T°: water temperature.

Species	Date and hour of recording	Country	Locality	Coordinates	Altitude	Air T°	Water T°
Chaparana (Paa) blanfordii (Boulenger, 1882)	05.05.1973 19h25-22h00	Nepal	Lam Pokhari	27°06'N, 87°59'E	2910 m	No data	No data
Chaparana (Paa) liebigii (Gunther, 1860)	25.06.1973 20h30	Nepal	Ghat	27°43'N, 86°43'E	2510 m	15.0°C	14.5°C
Chaparana (Paa) minica (Dubois, 1975)	03.08.1977 22h50	India (Himachal Pradesh)	Katrain	32°08'N, 77°09'E	1530 m	21.0°C	24.0°C
Chaparana (Paa) polunini (Smith, 1951)	23.06.1973 20h20	Nepal	Thammu	27°49'N, 86°41'E	3360 m	12.5°C	11.5°C
Chaparana (Paa) rostandi (Dubois, 1974)	21.08.1972 23h00	Nepal	Kalopani	28°38'N, 83°36'E	2540 m	16.0°C	No data
	04.09.1972	"	"	"	"	14.5°C	11.5°C
	31.08.1972 20h40-21h00	"	Kutsab Terna Tal	28°46'N, 83°43'E	2890 m	16.5°C	19.0°C
Chaparana (Paa) vicina (Stoliczka, 1872)	10.07.1977	India (Jammu & Kashmir)	Patnitop	33°02'N, 75°20'E	2050-2060 m	19°C	No data



Fig 1 *Chaparana (Paa) blanfordii* (Boulenger, 1882) female MNHN 1975 1056, Chauki (East Nepal), 15 July 1973

C minica 1989 2057-2058, 1989.2060, 1989.2062, 1989 2064-2067, 1989.2069-2070, 1989.2072, 1989 2075, 1989 2077-2083, (4) *C polunini* 1975 1441-1446, 1975 1457-1458; (5) *C rostandi* (Kalopani), 1975 959, 1975.962-964; *C rostandi* (Kutsab Terna Tal): 1973 310, 1973.320-321; (6) *C vicina*: 1985.1047.

Dates and times of recordings are provided in tab. 1, together with the data on temperature (air and water) when available.

Results

Chaparana (Paa) blanfordii (Boulenger, 1882)

Chaparana blanfordii (fig. 1) was recorded during the rainy season in a humid forest rich in small torrents, where calls could be heard all around both during day and night. The call was recorded at night in a small torrent running under trees. The males were calling from the bed of the forest torrent hidden between rocks, so not directly from the ground. Several males were calling widely spaced in this torrent

The call of this species (fig 2, tab 2) is short, high-pitched and repetitive It consists in continuous series of 15-17 short amplitude modulated notes with two lobes (0 026 s

Table 2. — Characteristics of the advertisement call of *Chaparana (Paa) blanfordii* and *Chaparana (Paa) liebigii* from Nepal, *Chaparana (Paa) minica* from India, *Chaparana (Paa) polunini*, *Chaparana (Paa) rostandi* and *Chaparana (Paa) vicina* from Nepal. Only one male was recorded for each species except for *P. rostandi* whose calls of three different males were recorded.
SVL, male snout-vent length; dc, duration of sequences from first to last note; dic, duration of silent intervals between two consecutive sequences; dn, duration of notes; din, duration of silent intervals between two consecutive notes; nn, number of notes per call; nns, number of notes per second; fib, frequence of the band *i*. Snout-vent lengths are expressed in mm, time measurements in seconds and frequencies in Hz. Value are given as: mean ± standard deviation, minimum-maximum, number of measurements.

Species	SVL	dc	dic	dn	din	nn	nns	f1b	f2b	f3b	f4b	f5b
Chaparana blanfordii (Lam Pokhari)	40.4 ± 2.0 38.5-42.5 3	3.96 ± 0.44 3.65-4.27 2	—	0.025 ± 0.009 0.010-0.044 32	0.236 ± 0.019 0.208-0.276 30	16.0 ± 1.4 15-17 2	4.1 ± 0.1 4.0-4.1 2	1529 ± 37 1469-1548 8	3076 ± 102 2860-3194 7	4631 ± 87 4487-4741 8	—	—
Chaparana liebigii (Ghat)	70.1 ± 9.7 56.4-89.5 11	2.26 ± 0.02 2.25-2.28 2	—	0.021 ± 0.003 0.016-0.026 31	0.130 ± 0.032 0.097-0.254 29	15.5 ± 0.7 15-16 2	6.9 ± 0.2 6.7-7.0 2	730 ± 52 686-784 9	—	—	—	—
Chaparana minica (Katrain)	34.5 ± [illegible]8 31.2-37.2 9	3.83 ± 0.91 3.0-5.5 6	—	0.087 ± 0.017 0.044-0.127 81	0.207 ± 0.061 0.124-0.466 72	13.5 ± 3.0 10-19 6	3.5 ± 0.2 3.3-3.8 6	1134 ± 43 1097-1215 20	2274 ± 78 2136-2410 20	3430 ± 116 3252-3625 17	4497 ± 116 4310-4643 10	5639 ± 116 5486-5780 9
Chaparana polunini (Thammu)	39.7 ± 2.3 37.3-43.6 8	1.06 ± 0.06 0.99-1.11 3		0.019 ± 0.007 0.003-0.030 31	0.093 ± 0.25 0.073-0.210 28	10.3 ± 0.6 10-11 3	9.8 ± 0.4 9.3-10.1 3	1642 ± 0 8	3274 ± 11 3260-3284 4	4954 ± 45 4902-4995 6	—	—
Chaparana rostandi (Kalopani)	5[illegible].7 ± 5.3 46.0-58.7 4	0.73 ± 0.1[illegible] 0.66-0.80 2	—	0.055 ± 0.006 0.046-0.061 7	0.216 ± 0.029 0.186-0.255 5	3.5 ± 0.7 3-4 2	4.8 ± 0.3 4.5-5.0 2	1593 ± 104 1371-1646 7	3138 ± 247 2645-3272 7	4686 ± 326 4056-4918 6	—	—
Chaparana rostandi (Kalopani)	51.7 ± 5.3 46.0-58.7 4	0.93 ± 0.15 0.83-1.04 2		0.054 ± 0.012 0.029-0.068 11	0.144 ± 0.016 0.127-0.177 9	5.5 ± 0.7 5-6 2	5.9 ± 0.2 5.8-6.1 2	1949 ± 165 1646-2077 11	3923 ± 378 3194-4212 9	6178 ± 117 6034-6289 6		
Chaparana rostandi (Kutsab Terna Tal)	49.8 ± 1.0 49.0-50.9 3	0.6	—	0.059 ± 0.005 0.055-0.065 4	0.131 ± 0.012 0.124-0.145 3	4	6.3	1641 ± 120 1469-1724 4	3262 ± 232 2919-3429 4	4883 ± 332 4389-5074 4	—	—
Chaparana vicina (Patnitop) MNHN 1985.1047	74.2	0.65 ± 0.12 0.47-0.88 10	5.06 ± 1.62 3.50-7.74 6	0.056 ± 0.021 0.015-0.108 69	0.054 ± 0.023 0.001-0.091 45	6.3 ± 1.0 5-8 11	10	350	700	—	—	—

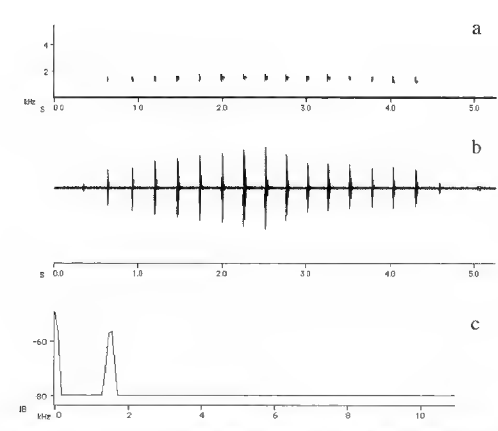


Fig. 2. A sequence of the advertisement call of *Chaparana (Paa) blanfordii* (Boulenger, 1882), Lam Pokhari (East Nepal), 5 May 1973: (a) spectrogram, (b) oscillogram, (c) spectrum of a note in the middle of the call.

in average) whose intensity increases until half or towards the end of the call, and then decreases in the same way. The average call duration is 4.0 s. The note repetition rate is quite slow (four notes per second). The intensity of each note decreases from its start to its end. Each note consists of two lobes, the first one with a large amplitude, the second one with a small one. The dominant frequency corresponds to the first frequency band and it is about 1529 Hz.

Chaparana (Paa) liebigii (Günther, 1860)

The recordings of *Chaparana liebigii* were made at night in the Dudh Kosi river bed. The male (fig. 3) could not be caught: it was calling hidden down between blocks close to water in this rather large torrent. The females were often seen close to the males, hidden in cavities between rocks.

Fig. 3. *Chaparana (Paa) liebigii* (Günther, 1860) male at night in its calling site under rock in the torrent's bed, Ghat (Center-East Nepal), 25 June 1973

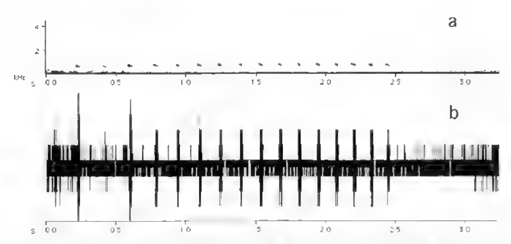


Fig. 4. A sequence of the advertisement call of *Chaparana (Paa) liebigii* (Günther, 1860), Ghat (Center-East Nepal), 25 June 1973, air temperature 15.0°C, water temperature 14.5°C. (a) spectrogram; (b) oscillogram. Note the important background noise due to the torrent



Fig. 5. – *Chaparana (Paa) minica* (Dubois, 1975): male MNHN 1989.2065, Katrain (Himachal Pradesh, India), 4 August 1977.

The call of this species (fig. 4, tab. 2) is a more or less regular succession of 15-16 short notes. It has the same structure as the call of *C. blanfordii* and lasts about 2.26 seconds. The note repetition rate is about 6.8 notes per second and the duration between two successive notes averages 0.134 s. The first note of the call is emitted with higher intensity than the following ones, and the duration of the interval between this note and the next one is longer than the interval between the other notes of the sequence. Because of the loud background noise, only the dominant frequency was measured (about 730 Hz).

Chaparana (Paa) minica (Dubois, 1975)

Chaparana minica (fig. 5) was recorded at night near a small stream where the males were calling, on the ground, in a basin filled with water. Among the six species studied in this paper, this is the only species in which two or three males were observed singing in a chorus.

Because of the problem encountered during recording (see *Materials and methods*), the values given here in text are rounded off, whereas the values in tab. 2 are reported as measured. The call of this species (fig. 6, tab. 2) consists of long (3.0-5.5 s) sequences, separated by intervals of about ten seconds, and composed of numerous notes (10-19). By its general aspect this call is quite similar to that of *C. liebigii*. Generally, the call of *C. minica* begins by one or

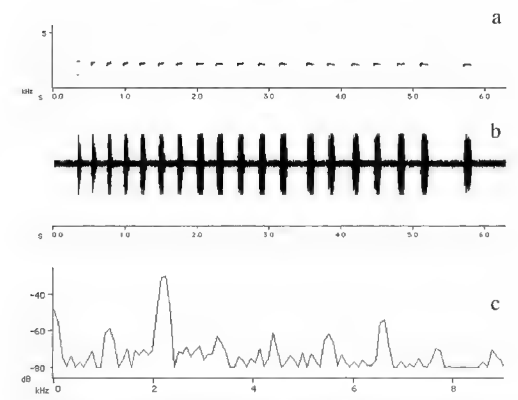


Fig. 6. – A sequence of the advertisement call of *Chaparana (Paa) minica* (Dubois, 1975), Katrain (Himachal Pradesh, India), 3 August 1977, air temperature 21.0°C, water temperature 24.0°C. (a) spectrogram; (b) oscillogram; (c) spectrum of a note in the middle of the call.

two notes shorter and greater in amplitude than the following ones. During the sequence the duration of notes increases (without variation in amplitude), as well as the duration of intervals between notes (this is not an artifact due to problems in the tape speed, as the same values are found in all recorded sequences). The signal stops usually with one or two notes emitted after an interval lasting in average 0.5 s (range 0.4-0.6), i.e., longer than the previous ones. The value of this unusual interval was excluded from the calculation of the mean interval between notes (din) (tab. 2). The amplitude of the notes reaches its maximum at its beginning then decreases gradually. Each note is composed of two parts, the first one, rounded, covering about the first quarter of the total length of the note, the second one, elongate, reaching its maximum amplitude shortly after its beginning, then decreasing slowly. An amplitude modulation, and a rising frequency modulation of about 300 Hz, are present at the beginning of each note. Up to nine frequency bands are visible in the spectrogram. Two frequency bands are more intense than the others: the second frequency band at about 2300 Hz corresponding to the dominant frequency, and the sixth band lying at about 6800 Hz.



Fig 7 *Chaparana (Paa) polunini* (Smith, 1951) male MNHN 1975 1454, Tesinga (Center East Nepal), 21 June 1973

Chaparana (Paa) polunini (Smith, 1951)

Calls of *Chaparana polunini* (fig 7) were heard in the afternoon, at 16h30, under a mossy block with water dripping, along a small steep torrent The calls started again at 19h00 and were recorded at 20h20

The call of this species (fig 8, tab. 2) is structurally similar to that of *C blanfordii*, i.e., with an increase of intensity of notes until middle of sequence, followed by a decrease in the same way It is composed of 10-11 short notes (about 0.020 s) separated by intervals of about 0.1 s. Each note consists of two parts, the former with a great amplitude and a rapid rise and fall, and the latter with a small amplitude The dominant frequency corresponds to the first frequency band and is about 1642 Hz. The second harmonic band is ill-defined. The dominant frequency of the third call (emitted by the same individual) is about 200 Hz lower and no harmonic bands are visible

Chaparana (Paa) rostandi (Dubois, 1974)

Chaparana rostandi was first recorded on 21 August 1972 near Kalopani in the large bed of the Kali Gandaki river The males (fig 9) were calling in smaller streams running under the

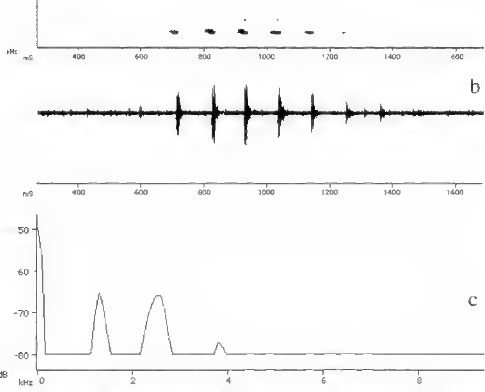


Fig 8 A sequence of the advertisement call of *Chaparana* (*Paa*) *polunini* (Smith, 1951), Thammu (Center-East Nepal), 23 June 1973, air temperature 12 5°C, water temperature 11 5°C (a) spectrogram; (b) oscillogram, (c) spectrum of a note in the middle of the call.

grassy bank of the river or in puddles under the banks left after decrease of the river level. The male recorded was hidden by grass and bush covering the banks. On 4 September 1972, in the same locality, a single male was calling and was recorded during night

C rostandi was recorded again on 31 August 1972 near the lake Katsab Terna Tal (type-locality of the species) at night. The males were calling in the water, under leaves and moss of the bank of the lake covered by vegetation, just below a small forest. Some males called on this bank but none was calling on or under the opposite bank lacking vegetation The males were spaced out from each other Other males called sporadically in the puddles, little streams and on the shore of the lake but their calls were not synchronized

The call of this species (fig 10, tab 2) is very short (shorter than or equal to 1 second) and composed of only a few whistled notes (from 3 to 6) The average note repetition rate is 5 7 notes per second The duration of notes is less than 0 06 s and the intervals between them are of moderate length (from 0 12 to 0 26 s) This call presents a remarkable peculiarity the last



Fig. 9. - *Chaparana (Paa) rostandi* (Dubois, 1974): male MNHN 1975.0964, Kalopani (Northwest Nepal), 22 August 1972.

note of each call has a very notable different tone (there are differences from 100 to more than 400 Hz between the dominant frequency of the last note and that of the others). Furthermore, this note is generally shorter and more damped than the previous ones. Its dominant frequency corresponds to the first frequency band and is about 1745 Hz.

Chaparana (Paa) vicina (Stoliczka, 1872)

The sequences of the advertisement call of *Chaparana vicina* (fig. 11) analyzed were emitted by a single male, hidden under a block in a torrent.

The call of this species (fig. 12, tab. 2) is composed of short sequences (0.65 s) emitted irregularly and separated by intervals of about 5 s. Each sequence consists of a series of 5-8 notes (mean length of note 0.056 s) separated by intervals of 0.05 s on the average. The structure of the notes is similar to that of the notes of *C. blanfordii*. The notes are composed of two bodies linked to each other. Their amplitude increases in the course of the sequence and then decreases very quickly in the last or two last notes. The dominant frequency is about 700 Hz and corresponds to the second band of the spectrogram. The fundamental frequency (or the first band) lies at about 350 Hz.

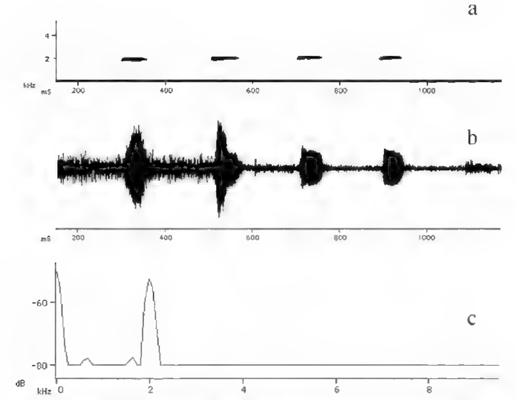


Fig. 10. A sequence of the advertisement call of *Chaparana (Paa) rostandi* (Dubois, 1974), Kalopani (Northwest Nepal), 4 September 1972, air temperature 14.0-14.5°C, water temperature 11.5°C. (a) spectrogram, (b) oscillogram, (c) spectrum of a note in the middle of the call. Note a fluctuation of the background noise during the call.

DISCUSSION

According to their general aspect, the signals of *C. blanfordii* and *C. liebigii* are close to each other, the signal of *C. polunini* appears to be similar to that of *C. vicina*, whereas those of *C. rostandi* and *C. minica* are particular. The calls of the two former species can be distinguished mainly by the values of the frequency bands. The advertisement calls of *C. polunini* and *C. vicina* share only a relatively high note rate (10 notes per second). The call of *C. minica* is particular by the fact that the amplitude of notes does not increase in the first part of sequence and does not decrease in the second half, only the two first notes have a greater amplitude than the others, whereas all the following ones have the same amplitude. The parameters of the calls of several individuals of *C. rostandi* recorded in two different populations show an important variation that covers the values found in other species. For instance the silent interval between the notes of a sequence varies from 130 to 250 ms and overlaps the values of *C. blanfordii* and *C. liebigii*. It is probable that a larger sampling of the



Fig. 11. – *Chaparana* (*Paa*) *vicina* (Stoliczka, 1872), male, Manali (Himachal Pradesh, India), 26 July 1977.

other species taken throughout their distribution area would also show a greater variation of each parameter.

Among these species, two are almost sibling species, *C. liebigii* and *C. vicina* (Dubois, 1976, 1980). Except for the male secondary sex characters, their morphologies are very similar, but their advertisement calls are quite different. The call of the former species is longer and composed of more notes than that of the latter species (more than two seconds with about 15 notes in *C. liebigii* in comparison to only half a second with 5-8 notes in *C. vicina*). Differences in notes duration (dn) and interval duration between notes (din) also exist between these two species, but an extended sample of calls could show an interspecific overlap for these parameters. Finally, there is a difference in the frequency, as *C. vicina* has a frequency band below the dominant frequency. Then, even if the two species have an identical dominant frequency, the call of *C. vicina* sounds lower-pitched.

Comparing the bands of dominant frequency of each species, we can notice that *C. liebigii* and *C. vicina* have a very low dominant frequency and *C. minica* a high dominant

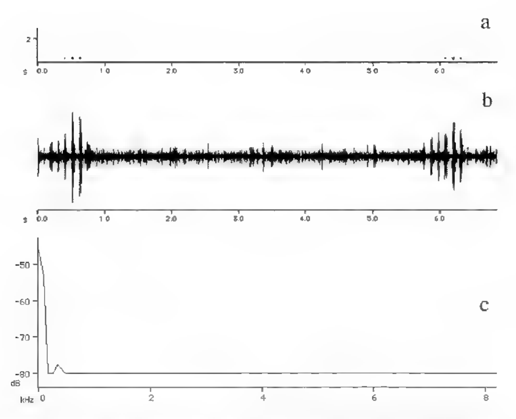


Fig 12 Two sequences of the advertisement call of *Chaparana Paa vicina* (Stoliczka, 1872), Patnitop (Jammu & Kashmir, India), 10 July 1977, air temperature 19°C: (a) spectrogram, (b) oscillogram, (c) spectrum of a note in the middle of the first sequence.

frequency, whereas the three other species cover the same frequency band, i e., about 1300 Hz to 1850 Hz This frequency band is surely the best suited to the ecological conditions of these species (see below). Among these three latter species, only *C polunini* and *C rostandi* are sympatric in a small area. Therefore other factors must allow the discrimination of the advertisement calls by the females The frequency of sound is not the only efficient parameter. The duration and shape of the call, the durations between the sequences and other parameters can play a part in the recognition of the specific signal too (PAILLETTE, 1971). The call of *C rostandi* is markedly different from the other two calls It has few whistle notes and the last note of each sequence is lower than the previous ones (from 100 Hz to 400 Hz lower pitched than the others). The first note is quite short, the notes in the middle of the call are the loudest and the last one is of lower intensity The differences between the calls of *C blanfordii* and *C*

polunini, which are not sympatric species although both present in Nepal and China (DUBOIS, 1979; FEI, 1999), are less pronounced.

Little information is available about the advertisement calls of species belonging to the other genera of the tribe Paini as redefined by OHLER & DUBOIS (2006). The advertisement call of *Quasipaa spinosa* (David, 1875) was briefly described as follows by POPE (1931): "(...) trilled base notes, a sound rivaling in volume and depth that of any frog I have ever heard. Another note is often emitted but it is a weak monosyllable and reminds one of the sound of a hammer brought down upon a small but long pipe filled with water". The call of *Quasipaa exilispinosa* (Liu & Hu, 1975) was briefly described from observations in captivity (VOITEL, 2000). It consists in a long series of notes (up to 30), each note lasting about 0.1 s. The value of the dominant frequency has not been specified though the area where frequencies are the most pronounced spreads from 320 to 1920 Hz. The advertisement call of a species of another genus, *Gynandropaa bourreti* (Dubois, 1987), was heard during a field course (GROSJEAN, pers. obs.). It consists in a series of pulse groups of equal intensity, lasting in total less than ten seconds. The genus *Chaparana* (or at least its subgenus *Paa*) seems to differ from the other genera by an advertisement call rather homogeneous in its gross features.

The species of the ranid tribe Paini are torrent-living species. Their calls are very different from those of other ranid species living in other habitats (DUBOIS, 1977*a-b*, DUBOIS & MARTENS, 1984; SUEUR, 1995) and share the same general characteristics. They are characterized by an important particularity: the notes are emitted with a low rate and in more or less long series (from 3 to 19 notes in the calls studied here) separated by relatively long intervals (from 3.5 seconds to one and a half minutes). The notes are very short (from less than 0.010 to 0.127 second) and are separated by short silent intervals (from juxtaposition of notes to 0.466 second). In spite of the weak intensity of calls, these features unquestionably allow a better distinction and localization among the noise of strong streams. This discrimination is likely to work mostly at the beginning and end of sequences.

The main characteristics of these calls have already been discussed (DUBOIS, 1977*a-b*; DUBOIS & MARTENS, 1984). Three major characteristics are common to these calls: (1) the calls are composed of short sequences of notes separated by long periods of silence; (2) the notes are pure, short, and have narrow frequency bands, (3) the notes are rhythmically separated within the sequences.

The calls of torrent-living species belonging to different anuran genera, such as the Oriental *Leptobrachella* (DRING, 1983*a*), *Leptolalax* (MATSUI, 1997), *Ansonia* (DRING, 1983*b*; INGER & DRING, 1988) and *Amolops* (MATSUI et al., 1993), present the same kind of temporal features. However, differences with the calls of *Chaparana* species also exist, especially in frequency features. Indeed the high dominant frequency of these torrent-living species spreads out on a large frequency band, whereas the calls of *Chaparana* exhibit a low dominant frequency limited to a narrow frequency band. This is found also in some Bolivian torrent-living frog traditionally referred to the genus *Hyla* (MÁRQUEZ et al., 1993), and placed in the genus *Hypsiboas* by FAIVOVICH et al. (2005). The concentration of energy in a very narrow frequency band, generally the lowest frequency, would provide a greater amplitude to the call and thus favour the propagation of the pure notes over a greater distance, and the signal reception may be more efficient against a background of wide band noise (CHAPPUIS, 1971; MORTON, 1975). However, the calls of the *Chaparana* remain of weak intensity and a single

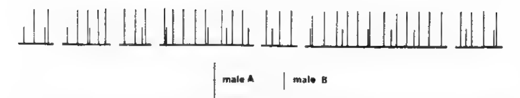


Fig 13 Diagram showing the succession of the calls of two males of *Chaparana (Paa) polunini* (Smith, 1951) calling close to each other at Thammu (Center-East Nepal), 23 June 1973 Each vertical line indicates the beginning of a sequence of call The time scale is arbitrary, based on the recorder counter The breaks on the horizontal line indicate stops in the recording (after DUBOIS, 1977*b*).

rock in the torrent interposed between the observer and the calling frog may be a sufficient obstacle to prevent the frog's call from being heard. The adult males of this genus are distributed along the torrents so that often only the call of a single male can be heard at once by a human observer. The localization of the male is helped in part by the slight binaural discrimination at beginnings and ruptures in the sound emission (MARLER, 1967; FENG et al., 1976, GRIBENSKI, 1977, DUBOIS & MARTENS, 1984, SUFUR, 1995), fast rise and fall times, short durations and moderate repetition rates (LITTLEJOHN, 1977). As a result, the calls of these frogs stand out well on the continuous background noise of the torrent (DUBOIS, 1977*a-b*; DUBOIS & MARTENS, 1984).

The other characteristics found in the *Chaparana* calls, such as a low dominant frequency, a prominent harmonic content and a rapid rise and fall of each note, are shared by some fossorial microhylid frogs' calls from New Guinea (MENZIES & TYLER, 1977) as well as by Australian and Chilean leptodactylid burrowing frogs (LITTLEJOHN & MAIN, 1959, PINGILLEY, 1971, FORMAS, 1985, PENNA & SOLÍS, 1999). These frogs exhibit a dominant frequency lower than 3200 Hz which suits well with the resonance of the burrow and so amplifies the efficiency of the call (BAILEY & ROBERTS, 1981). Other anurans that call hidden from within the ground, such as species belonging to the genera *Alytes* or *Tomodactylus* (DIXON, 1957; MÁRQUEZ & BOSCH, 1995), also share tonal calls with the *Chaparana* species. When the torrents are not in spate, the *Chaparana* call hidden under the rocks. This calling site can be compared to the burrows and subterranean habitats of fossorial frogs. Their notes present a dominant frequency band comprised between 700 and about 2000 Hz (excluding the value of *C. minica*). In all the species studied here, except for *C. minica* and *C. vicina*, the dominant frequency is the fundamental too. The call of *C. minica*, species less rheophilous than the others, which calls from the side of the stream, has a higher dominant frequency

In most anuran species, during calling period the males synchronize their calls just as if they answered each other Some species arrange their calls in chorus. This is the case in the European species of the genus *Hyla*, particularly in the well-studied *Hyla meridionalis* (PAILLETTE, 1970, 1976). On the contrary, the males of *Chaparana* studied above, except in *C. minica*, do not answer each other DUELLMAN (1967) defined different categories of calling behaviour in the light of the social organization of frogs, and named the latter calling behaviour "individual" This behaviour was observed in the field in *C. polunini* (DUBOIS,

1977*b*) and *C. rostandi*, and in playback experiments in *C. blanfordii*. A diagram showing the succession of the call sequences of two males of *C. polunini* that were calling close to each other illustrates this phenomenon (fig. 13). Territorial calls, frequent in paddy-field species and sometimes in forest species (DUBOIS, 1977*a*), do not seem to exist in the species of this genus. In standard conditions, the males are distributed far from each other, so that only the call of one individual can be heard at once by a human observer. The calls of different males may interfere when the torrents are high, so that the usual shelters of the frogs are flooded. The advertisement calls can have a territorial function so that when a male hears the call of another male he could move away until not hearing it anymore. This function of call has also been assumed in the Papuan microhylids (MENZIES & TYLER, 1977).

The call of *C. minica* has the highest dominant frequency of all of the calls analyzed until now, and numerous harmonics. Furthermore, in this species (but also in *C. vicina*), the dominant frequency corresponds to the second frequency band (to the first band in the other four species). These differences with the calls of the other species could be due to the slightly different mode of life of this species. *C. minica* inhabits rather quiet small streams rather than violent torrents and does not call hidden under rocks but on the side of the stream, in puddles. This may explain why the energy of call is not concentrated on a narrow frequency band but spread over a wide range. Furthermore the shape of the advertisement call shows more similarities to the calls of the species inhabiting open areas and some typical features of calls adapted to torrents, such as short duration of notes or variation of amplitude of notes during the call, are not present. Another striking difference to other members of the genus reviewed here is the presence of a chorus like in species living in open areas. So *C. minica* has a habitat and a calling behaviour slightly different from those of the other species considered in this paper. However its call still possesses several features adapted to communication in torrents.

As suggested by DUBOIS (1977*b*), the strong similarities which exist between the calls of the species studied here more probably express the phylogenetic relationships of these species rather than an evolutive convergence. The constraint imposed by the habitat was presumably the predominant factor responsible for the elaboration of such call features which then were conserved through speciation. A study on American bufonids and hylids (COCROFT & RYAN, 1995) showed that several call parameters can be conserved through repeated speciation events within a homophyletic group.

RÉSUMÉ

Les chants de six espèces du genre *Chaparana* (sous-genre *Paa*) sont décrits, deux d'entre-eux (*Chaparana minica* et *Chaparana vicina*) pour la première fois. Pour chacune des espèces, de nombreux paramètres de durée et de fréquence sont donnés. Chaque chant est illustré par un oscillogramme, un sonagramme et un spectre. Les caractéristiques générales de ces chants particuliers sont considérées comme des adaptations à un environnement torrenticole. Les chants et modes de vie de ces grenouilles concordent avec les taxons définis par des caractères morphologiques et moléculaires.

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Hyla reinwardtii Schlegel, 1840 as a nomen protectum

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Following article 23.9.1 of the *International Code of Zoological Nomenclature*, the nomen *Rhacophorus moschatus* Kuhl & Van Hasselt, 1822 should be considered a nomen oblitum and the nomen *Hyla reinwardtii* Schlegel, 1840, its junior subjective synonym, should be treated as the valid nomen of the Reinwardt's Gliding Frog. In order to demonstrate the large acceptance of the nomen *Rhacophorus reinwardtii*, we provide a list of references using this nomen as valid. A lectotype is designated for *Hyla reinwardtii* Schlegel, 1840 and its description and figure are provided.

South east Asia is one of the hot-spot areas of amphibian biodiversity (STUART et al., 2004). Many groups of frogs of this region have not been revised recently, and in those which were so many new species were described (e.g. VEITH et al., 2001; BROWN & GUTTMAN, 2002; OHLER, 2003). As many old scientific names or *nomina* (DUBOIS, 2000) are "sleeping" in the synonymies of many species, in order to link new results of research to previous knowledge, reliable nomenclatural work should be done prior to naming new taxa.

We have shown on several occasions how useful the Principle of Priority is for automatic determination of the valid nomen of a taxon in case of synonymy (DUBOIS & OHLER, 1995, 1997, 1999, 2000, DUBOIS, 1995, 1998; OHLER & DUBOIS, 1999, BOSSUYT & DUBOIS, 2001). We expressed our disagreement with some of the decisions of the International Commission on Zoological Nomenclature giving precedence to a nomen that had been used only in a few more publication than a senior synonym, although in some of these cases "usage" of the protected nomen had been limited to specialised taxonomic publications (DUBOIS, 2005*a-c*). We always strongly insisted and continue to insist that such cases should not be concerned by reversal of precedence as they only tend to weaken the legislative value and strength of the *Code* in the eyes of zoologists and thus contribute to spreading arbitrary and chaos in zoological nomenclature. Nevertheless there are cases when such an act is a reasonable one. In the edition of the *Code* currently in force (ANONYMOUS, 1999), Article 23.9 gives rules for reversal of precedence in such cases.

DUBOIS (1982, 1989) pointed to some problems in relation to the genus-group nomen *Rhacophorus* Kuhl and Van Hasselt, 1822 and the species-group nomina *Rhacophorus mos-*

chatus Kuhl & Van Hasselt, 1822 and *Hyla reinwardtii* Schlegel, 1840. When creating the genus-group nomen *Rhacophorus* for large tree-frogs from Java, Kuhl & Van Hasselt (1822*a*) referred two specific nomina to this genus. The first nomen, *Rhacophorus reinwardtii*, was not accompanied by any description, definition or indication, and consequently must be considered a nomen nudum (Dubois, 1989). This specific nomen became only available in the work of Schlegel (1840) who figured this tree-frog species as *Hyla reinwardtii*. The second specific nomen proposed by Kuhl & Van Hasselt (1822*a*), *Rhacophorus moschatus*, was accompanied by a very short indication ("dewijl zij eenen sterken Bisamreuk zeer ver verspreidt", i.e., "because it spreads a strong musky scent very far") which is sufficient to make the nomen *moschatus* nomenclaturally available as of Kuhl & Van Hasselt (1822*a*). This nomen being the only available specific epithet associated with the generic nomen *Rhacophorus* in the original description of the genus, *Rhacophorus moschatus* Kuhl & Van Hasselt, 1822 is the type-species by monotypy of *Rhacophorus* Kuhl & Van Hasselt, 1822 (Dubois, 1989).

The status of the species group nomina *Rhacophorus moschatus* Kuhl & Van Hasselt, 1822 and *Hyla reinwardtii* Schlegel, 1840 remains to be dealt with Brongersma (1942) gave arguments to support the opinion that *Rhacophorus moschatus* was proposed for a juvenile of the species known as *Rhacophorus reinwardtii* If this is true, the two species-group nomina are synonymous, and the valid nomen should be the senior one. But the junior synonym, *Hyla reinwardtii*, has been widely used in the combination *Rhacophorus reinwardtii*, and, to our knowledge, *Rhacophorus moschatus* has never been used as a valid nomen. Application of the Principle of Priority would lead to disturbance of a usage established for almost 200 years, including in popular and non-specialised taxonomic literature. The case was submitted to the International Commission on Zoological Nomenclature 20 years ago (Dubois, 1989. 101), but despite the rare clarity of the case this application was never published in the *Bulletin of Zoological Nomenclature* and no vote was ever organised on this question (Dubois, 1989). Working on a list of synonymy of Oriental amphibians we reconsidered this case under the new edition of the *Code*. This text shows an important novelty regarding the rules regulating change of precedence between synonymous nomina. The way this rule is formulated (especially mentioning "valid" rather than "available" nomina) is highly open to criticism (Dubois, 1999, 2005*b*-*c*), and changes in this writing should be considered in the future. Nevertheless, in the present case, this rule allows to establish the valid nomen of the species at stake without having any more to wait for an improbable vote of the Commission.

Article 23 9 1 gives the conditions when prevailing usage must be maintained "the senior synonym or homonym has not been used as a valid name after 1899" (Article 23 9.1.1), and "the junior synonym or homonym has been used for a particular taxon, as its presumed valid name, in at least 25 works, published by at least 10 authors in the immediately preceding 50 years and encompassing a span of not less than 10 years" (Article 23 9 1.2) In order to apply Article 23 9 1, an author must cite the two nomina together and state explicitly that the junior nomen is valid and that the action is taken in accordance with this Article In particular it must be stated that Article 23.9.1 1 applies and that conditions of Article 23 9 1 2 are met

Considering the usage of the nomina *Rhacophorus moschatus* and *Hyla reinwardtii*, the conditions of Article 23 9 are clearly met for both nomina The nomen *Rhacophorus moschatus* has never been used as valid nomen for these tree-frogs; all authors who mentioned this

nomen considered it as invalid (DUBOIS, 1982, 1989, FROST, 1985, ZHAO & ADLER, 1993). On the other hand, *Hyla reinwardtii* (as *Rhacophorus reinwardtii*) has been used largely, in particular in faunal lists, field guides, books on amphibian biology and general zoology. This species is well-known also by non-specialists, as it is one of those that have a particular mode of aerial locomotion, gliding in the canopy of primary forests. A list of 25 publications, by 25 independent authors (sensu DUBOIS, 2005*c*), citing the nomen *Rhacophorus reinwardtii*, is provided in Appendix 1. Among hundreds, these references were chosen in order to represent a great variety of countries and of works, to corroborate large acceptance.

Having met conditions given in Article 23.9 of the *Code*, the nomen *Hyla reinwardtii* has precedence over *Rhacophorus moschatus*. This action only considers precedence but not availability in the case where synonymy of both nomina should be questioned. As a matter of fact, some authors (VAN KAMPEN, 1923: 254, AHL, 1931: 148, WOLF, 1936: 187) suggested that *R. moschatus* might be the species later called *Hyla margaritifera* Schlegel, 1844, and also *Hyla javanus* Boettger, 1893. In such a case the nomen *Rhacophorus moschatus* would remain available for possible "resurrection", as is explicitly stated in Article 23.9.2. Stabilisation of the status of this nomen would require designation of a neotype, as the original syntypes are lost (BRONGERSMA, 1942).

In the publication where the nomen *Hyla reinwardtii* was made nomenclaturally available, SCHLEGEL (1840) provided figures of three specimens, thus pointing to morphological and color variation in this group. CHAN-ARD et al. (1999) also documented this variation, as they showed a photo of a specimen which they only tentatively recognized as being a member of *R. reinwardtii*. Should this variation reflect specific differentiation, the nomen *Rhacophorus moschatus* could possibly be available for one of the taxa. A modern revision of the species group using etho-ecological, genetic and molecular characters might redefine species limits. In this perspective, it is important to stabilise the nomenclatural status of the nomen *Hyla reinwardtii* Schlegel, 1840. As the nomen is available from the *Abbildungen*, only the specimens originally illustrated in the latter are syntypes. These specimens are still extant and kept in the collections of the Nationaal Natuurhistorisch Museum (formerly Rijksmuseum van Natuurlijke Historie), Leiden, Netherlands (RMNH). Plate 30 of SCHLEGEL (1840) shows three specimens: figures 1 and 2 correspond to RMNH 6517 A, figure 3 to RMNH 3899 and figure 4 seems to be painted on the model of RMNH 1970 A. Only these three specimens are syntypes of this nominal species, and not the two additional specimens in the Leiden Museum listed by FROST (1985: 547) as syntypes (RMNH 1870.B and 6517 B). We hereby designate the specimen RMNH 6517 A as lectotype. This choice is justified as only this specimen in SCHLEGEL's plate (1840) clearly corresponds to the current concept of the species. This specimen is from Java, so there is a type-locality indication. We provide below in Appendix 2 a description and a photograph (fig. 1) of this specimen.

For the time being, the synonymy of *Rhacophorus reinwardtii* is as follows.

Rhacophorus reinwardtii (Schlegel, 1840)
[Reinwardt's Flying Frog, Green Flying Frog, Black-webbed Treefrog]

Rhacophorus moschatus Kuhl & Van Hasselt, 1822*a*: 104. – **Nomen oblitum**. **Onomatophore**: syntypes unknown. **Type-locality**: near Rosamelen forest, region of Gunung

Pangerango (106°57'E, 06°46'S), near Bogor [Buitenzorg], Java, Indonesia. **Synonymy**: BRONGERSMA (1942: 345). – **Comments**: BRONGERSMA (1942) considered the specimen of figure 4 in SCHLEGEL (1840) as one of the syntypes of this nominal species. This specimen closely resembles in color pattern RMNH 1970 A, which cannot be a syntype as it has not been collected by Kuhl but by S. Müller in Sumatra, according to the RMNH catalogue.

"*Rhacophorus reinwardtii*" Kuhl & Van Hasselt, 1822*a*: 104. – **Nomen nudum.**

"*Rhacophorus rheinwardtii*" Kuhl & Van Hasselt, 1822*b*: 476. – **Nomen nudum.**

"*Hypsiboas reinwardtii*" Wagler, 1830: 200. – **Nomen nudum.**

Hyla reinwardtii Schlegel, 1840: 105. – **Nomen protectum.** – **Onomatophore**: lectotype, by present designation (see Appendix 2 below), RMNH 6517.A, adult female. – **Type-locality**: Java, Indonesia.

Rhacophorus reinwardtii: DUMÉRIL & BIBRON, 1841: 532.

Polypedates reinwardtii: SIEDLECKI, 1909: 704.

Rhacophorus reinwardti: VAN KAMPEN, 1910: 43.

R[hacophorus] (R[hacophorus]) reinwardtii: AHL, 1931: xii, 60, 171.

Rhacophorus (Rhacophorus) reinwardtii: DUBOIS, 1987: 77.

? *Rhacophorus reinwardti* var. *lateralis* Werner, 1900: 495 [nec *Rhacophorus lateralis* Boulenger, 1883: 162]. – **Onomatophore**: holotype, Naturhistorisches Museum, Basel, Switzerland (NHMB) 1192, adult female (FORCART 1946: 132). – **Type-locality**: Batu Bara, Laut Tador, Sumatra, Indonesia. – **Synonymy**: WOLF (1936: 213).

RÉSUMÉ

En raison de l'article 23.9.1 du *Code International de Nomenclature Zoologique*, le nomen *Rhacophorus moschatus* Kuhl & Van Hasselt, 1822 doit être considéré comme un nomen oblitum et le nomen *Hyla reinwardtii* Schlegel, 1840, son synonyme subjectif plus récent, comme le nomen valide de la Rainette parachute de Reinwardt. Une liste de références de travaux dans lesquels le nomen *Rhacophorus reinwardtii* est employé comme nomen valide permet de démontrer l'importante utilisation de ce nomen. Un lectotype est désigné pour ce nomen et sa description et figure sont données.

ZUSAMMENFASSUNG

Aufgrund des Artikels 23.9.1 des *International Code of Zoological Nomenclature* sollte der Name *Rhacophorus moschatus* Kuhl & Van Hasselt, 1822 als nomen oblitum betrachtet werden und der Name *Hyla reinwardtii* Schlegel, 1840, sein jungeres subjektives Synonym, sollte der valide Name des Reinwardtschen Flugfrosches sein. Eine Liste von Werken, in denen der Name *Rhacophorus reinwardtii* als valider Name gebraucht wird, soll die breite Anerkennung des Namens bezeugen. Ein Lectotypus für diesen Names wird designiert und seine Beschreibung und Abbildung werden gegeben.

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APPENDIX 1

LIST OF 25 REFERENCES OF GENERAL WORKS USING THE NAME *RHACOPHORUS REINWARDTII*

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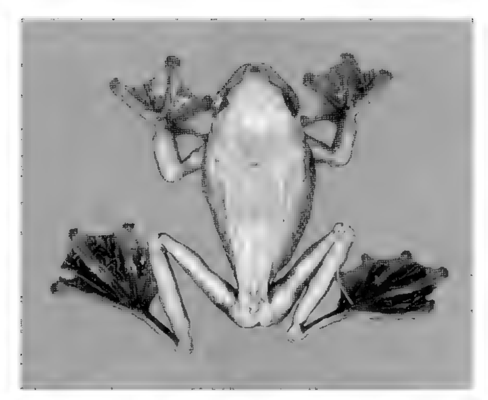


Fig 1 Lectotype of *Hyla reinwardtii* Schlegel, 1840, RMNH 6517 A, in dorsal view

Appendix 2

Description of lectotype of *Hyla reinwardtii*

To facilitate comparisons, the format of this description is the same as in our other recent descriptions of Oriental Amphibia, especially of the genus *Rhacophorus* (Ohler & Delorme, 2006). Measurements were taken in mm They are designated by the following abbreviations SVL: snout vent length *Head*: HW: head width, HL: head length (from back of mandible to tip of snout); MN distance from back of mandible to nostril, MFE: distance from back of mandible to front of eye, MBE: distance from back of mandible to back of eye; IFE: distance between front of eyes; IBE distance between back of eyes, IN internarial space, EN distance from front of eye to nostril, EL: eye length, SN: distance from nostril to tip of snout, SL: distance from front of eye to tip of snout; TYD greatest tympanum diameter, TYE: distance from tympanum to back of eye; IUE minimum distance between upper eyelids, UEW maximum width of inter upper eyelid. *Forearm*: HAL hand length (from base of outer palmar tubercle to tip of toe), FLL forelimb length (from elbow to base of outer tubercle), TFL third finger length (from base of first subarticular tubercle), pa1-pa4 width of pads of finger I to IV, waI-waIV: width of fingers I to IV. *Hindlimb*: FL: femur length (from vent to

knee), TL: tibia length; FOL, foot length (from base of inner metatarsal tubercle to tip of toe); FTL· fourth toe length (from base of first subarticular tubercle to tip of toe), ppI-ppV: width of pads of toes I to V; wpI to wpV· width of toes I to V; IMT: length of inner metatarsal tubercle; ITL inner toe length. *Webbing*: MTTF. distance from distal edge of metatarsal tubercle to maximum incurvation of web between third and fourth toe; TFTF. distance from maximum incurvation of web between third and fourth toe to tip of fourth toe; MTFF: distance from distal edge of metatarsal tubercle to maximum incurvation of web between fourth and fifth toe, FFTF. distance from maximum incurvation of web between fourth and fifth toe to tip of fourth toe).

Lectotype of *Hyla reinwardtii* Schlegel, 1840, by present designation, RMNH 6517 A, adult female (fig. 1). Poor preservation, specimen stuffed and dried.

(A) Size and general aspect (1) Specimen of moderate size (SVL 69.3 mm), body rather robust.

(B) Head. (2) Head moderate, as long (HL 23.4 mm) as wide (HW 23 3 mm; MN 19.5 mm; MFE 15.7 mm; MBE 8.4 mm), flat. (3) Snout rounded, not protruding; its length (SL 10 53 mm) longer than horizontal diameter of eye (EL 9.47 mm) (4) Canthus rostralis rounded, loreal region convex; obtuse in cross section. (5) Interorbital space convex, larger (IUE 6.84 mm) than upper eyelid (UEW 5.26 mm) as large as internarial distance (IN 6.79 mm); distance between front of eyes (IFE 14.1 mm) about two thirds of distance between back of eyes (IBE 21.9 mm). (6) Nostrils rounded, without flap of skin; as close to tip of snout (NS 5.93 mm) as to eye (EN 5 66 mm). (7) Pupil indistinct (8) Tympanum (TYD 5.53 mm), distinct, oval, oblique; tympanum-eye distance (TYE 0 92 mm) one fifth its diameter. (9) Pineal ocellus absent (10) Vomerine ridges not observed (11) Tongue not observed. Tooth-like projection on maxilla absent

(C) Forelimbs. (12) Arm rather short, thin, fore-arm (FLL 13.7 mm) shorter than hand (HAL 22 1 mm), not enlarged. (13) Fingers I and II rather long, thin; fingers III and IV long and thin (TFL 12.4 mm). (14) Relative length, shortest to longest. I <II < IV <III. (15) Tips of fingers I to IV rounded, enlarged, circum-ventral discs on fingers I to IV, very wide compared to finger width (paI 2 92 mm, waI 1.56 mm, paII 3 76 mm, waII 1 94 mm, paIII 4.15 mm, waIII 2 59 mm, paIV 4.41 mm, waIV 2 40 mm). (16) Fingers with webbing: I 2 – 1 II 0 0 III 0 0 IV (17) Subarticular tubercles present, poorly distinct, rounded, single, proximal tubercle of fingers III and IV small and flat. (18) Prepollex oval, very prominent; palmar tubercle indistinct.

(D) Hindlimbs. (19) Shank six times longer (TL 32.8 mm) than wide (TW 5.3 mm), shorter than thigh (FL 35 5 mm) but as long as distance from base of internal metatarsal tubercle to tip of toe IV (FOL 33.0 mm) (20) Toes long, thin, toe IV (FTL 17.9 mm) longer than third of distance from base of tarsus to tip of toe IV (TFOL 48.2 mm) (21) Relative length of toes, shortest to longest. I < II < V < III < IV (22) Tips of toes rounded, enlarged, circum-ventral grooves on toes I to V (ppI 2.40 mm, pwI 1 30 mm; ppII 2 27 mm, pwII 1 62 mm, ppIII 2 98 mm, pwIII 1 94 mm, ppIV 3 50 mm, pwIV 1 94 mm, ppV 2 98 mm, pwV 1 94 mm) (23) Webbing complete I 0 0 II 0 0 III 0 – 0 IV 0 0 V (MTTF 23.1 mm, MTFF 25 5 mm, FTFT 7 8 mm; FFTF 13 4 mm) (24) Dermal fringe along toe V from tip of toe along toe, continuing on tarsus to heel, well developed (25) Subarticular tubercles present, distinct, rounded, simple, al. present. (26) Inner metatarsal tubercle short, distinct, its length

(IMT 2.50 mm) 4.1 times in length of toe I (ITL 10.26 mm). (27) Tarsal fold absent. (28) Outer metatarsal tubercle, supernumerary tubercles and tarsal tubercle absent.

(E) Skin (29) Dorsal and lateral parts of head and body smooth, flanks with small glandular warts getting larger ventrally. (30) Dermal folds on forearm, heel, tarsus, metatarsus and vent; latero-dorsal folds absent; "Fejervaryan" line absent; lateral line system absent; supra-tympanic fold absent; cephalic ridges absent; co-ossified skin absent. (31) Dorsal parts of limbs smooth. (32) Ventral parts of head, body and limbs. throat and chest smooth; belly and thigh covered with treefrog belly skin. (33) Macroglands absent.

(F) Coloration in alcohol (34) Dorsal and lateral parts of head and body. dorsal parts of head and body and upper part of flank creamy white; lower part of flank brown with whitish spots corresponding to glandular warts; loreal region, upper lip, tympanic region and tympanum creamy white. (35) Dorsal parts of limbs creamy white; posterior part of thigh brown. (36) Ventral parts of head, body and limbs: throat, margin of throat and chest white, belly and thigh brown with white spots corresponding to glandular warts; webbing between toes I and II creamy white; other toes dark brown with whitish longitudinal bands.

(G) Secondary sexual characters. Not observed.

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The suburban common frog (*Rana temporaria*) population in the eastern Helsinki suburb, Finland

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The common frog population in the eastern Helsinki suburb was estimated in 1999-2002 by counting the numbers of egg clumps in spawning sites. The study area covered 1590 ha. The frogs were found in various types of green areas such as woodlands, agricultural land and various types of parks. These areas covered 40 % of the whole study area. The population was 786 ± 262 spawning female frogs. The population size increased during the study period. The population density was 1.3 females/ha in green areas. Spawning sites were small dikes, ponds, brooks and their wider parts with still water. Small and shallow dikes and ponds are vulnerable to overfilling and other negative changes. It was found that some new sites were made as a by-product of city works. The amount of spawning habitats seemed to be the density dependent limiting factor controlling the common frog population.

INTRODUCTION

It has been repeatedly reported that frog populations are especially sensitive to various types of pollution and habitat destruction. All species are not, however, as sensitive. Several healthy populations have been monitored long enough to verify this (e.g., MEYER et al., 1998). It has been stated that the common frog is less sensitive to urbanisation than many other species (KUZMIN, 1994) and seems to survive in urban conditions (HITCHINGS & BEEBEE, 1997).

Frog population studies such as catching and recatching are time consuming. HAAPANEN (1982) has developed for northern habitats a counting procedure which gives quite exact numbers of spawning female common frogs. The same type of method to estimate the population and to observe the annual variation of a frog population in a long term has been used e.g. by KUTENKOV & PANARIN (1995). Although males and subadults are ignored, the spawning females are the essential part of the population.

The aim of this study is to count the number of breeding female common frogs and to describe the habitats in suburban conditions in the boreal zone.

Study area and methods

The common frog populations were surveyed in the eastern part of Helsinki (60°12'N, 25°08'E, 0-25 m above sea level). The study area covers about 1590 ha, which is 8.5 % of the whole area of Helsinki (fig. 1). The area consists of apartment house sectors, small house blocks, industrial areas, and various kinds of traffic lines and green areas (forest, park and meadow in fig. 1). The local industry does not pollute air or waters. The acid rain load has been cut down by 60 % from the situation in the late 1970's (Kulmala et al., 1998). In the late 1980's several lichen species have reinvaded the region showing the enhanced air quality. One apartment house block has been constructed on an old dumping place in the 1970's. In the late 1990's it was found to pollute the soil and small dikes below. According to the information from the City of Helsinki, this pollution is limited to the nearby dikes and does not reach the study area itself.

The constructed areas outside the green areas cannot be regarded significant as a common frog habitat because of high density of traffic lines, the blocking effect of houses on the migration and only minimal green areas. Therefore those areas have not been included in this study as a common frog habitat.

The study area was sparsely inhabited, in some places like countryside, until the 1960's. The rapid urbanisation took place in the 1960's and 1970's, including four-lane road and underground railway constructions. The present green areas appear in the city general plan mostly as parks, outdoor recreation areas and as a university farm. The green areas have been more or less the same during the last 25-30 years. Altogether there are 633 ha green areas, which were divided into 24 sub-areas. These are isolated from each other in most cases by streets, four-lane highways or house blocks.

The green areas are most extensive in the western part of the study area in the university farm, where they form 50 % of all green areas. The green areas altogether cover 40 % of the whole study area but only 25 % east of the university farm (fig. 1).

These green areas were classified into five different habitat types as follows: (1) broad leaved woodlands with rich natural field layer vegetation, later called woodlands; (2) woodland parks; (3) areas covered partly with woodlands and partly well-managed short-cut lawns, called semi-open areas below; (4) agricultural areas; and (5) barren rocky pine woods. The rocky barren woodlands are mostly 20-25 m above sea level, often with fairly steep slopes. The other habitat types are found mostly in lowlands.

There are three brook watershed areas in the study area (fig. 1). The total length of brooks in the study area is 11 km. These brooks have been canalised in earlier times. Parts of them have been restored in recent years. In addition there are small ponds and dikes. Some of the dikes date back to old farming which has ceased decades ago. The few pH measurements in these brooks show that water is close to neutral during the spawning season. The observed pH values are 6.5-7.3 and the water quality in general was good in surveys made by the City of Helsinki (Jalava, 1987; Ketola, 1998).

The dikes are very small. The amount of water may be only some cubic metres and most of them are dry later in the summer. Only a small proportion of them have water plants. The

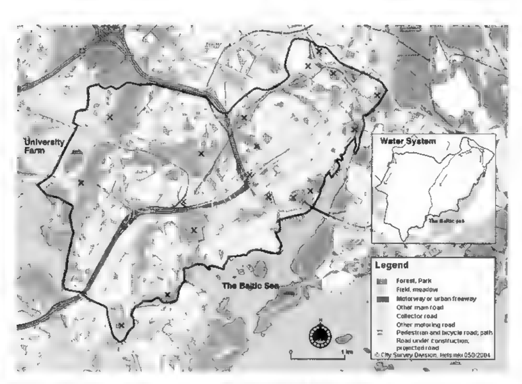


Fig. 1. The study area in the eastern part of the City of Helsinki. X-marked areas show the green areas, where spawning frogs were found. Constructed areas, motorways and other main roads isolate these from each other. Two crosses seem not to be in green areas, but this is not the case. These green areas are either in the corner of an industrial area or above the subway where natural vegetation with a pond has survived. The green areas are not connected to rural areas. The index map shows the brook systems with flowing directions one on the western border, one in the middle and one on the eastern side of the study area.

small ponds are bigger, from 100 to 1000 m^2, 20-60 cm deep. Many of them are permanent water bodies. Brooks and their wider parts are permanently wet at least in normal years. Only such parts of brooks are used by frogs where there is still water during the spawning season. These brooks are small, 0.5-2 m wide and 20-50 cm deep. Measurements of the flow from one brook were 1.5-1280 l/s, with an average of 35 l/s (KETOLA, 1998). During spawning the flood is mostly over but the flow is apparently above the average.

The study area is connected to the Baltic Sea. The brackish waters are not used as breeding habitat (HAAPANEN, 1982), although the salt content is hardly noticeable. Moreover it varies greatly (0-0.5 ‰), depending on the amount of fresh water and winds.

The spawning sites are of four different types: dikes, small ponds, brooks with still water and the wider parts of brooks with still water.

The counts were made over four years (1999-2002). The year 2002 was exceptionally dry. The amount of rain from early April to mid-June was only 72 mm or 56 % of the long term average. The other years were wet or close to the normal.

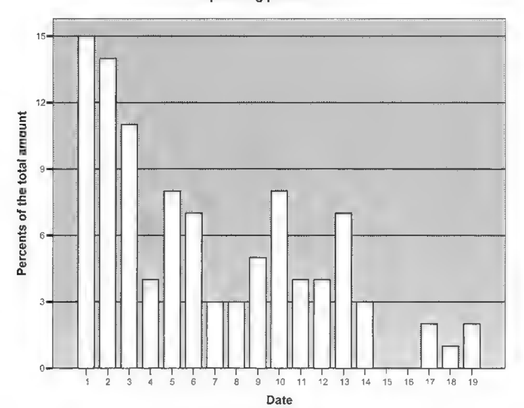


Fig. 2. - The spawning pattern of the common frogs in 2002. The columns show the percentage of egg clumps laid each day (*n* = 192). The spawning started April 14 and took 19 days.

The census of the common frog population is based on the counting of egg clump masses during the breeding season. One female lays only one egg clump per season (Savage, 1961). The census followed the procedure proposed by Haapanen (1982). This method was developed further, as follows. It is impossible to make the census when all the clumps have to be surveyed at once, as the development starts immediately after laying and accumulations of dozens of egg clumps can occur. However, each day the newly appeared egg clumps can be distinguished and counted in these egg clump groups (Haapanen, 1982). In 2001 and 2002 a certain number of breeding sites were surveyed daily from the early beginning of breeding until no new egg clamps were seen. So it was possible to see which percentage of the total egg clump numbers had been laid each day of the census period (fig. 2).

The spawning sites surveyed daily made a fairly representative sample of the total spawning female population as they formed 10 and 19 of the total census in 2001 and 2002, respectively. The census in other spawning sites was made approximately after 10 days from

the start of egg laying or somewhat later and the numbers were corrected using the correction figure based on the results in areas followed daily (fig. 2) In 1999 and 2000 only one census was made with no additional counting as the method would require (HAAPANEN, 1982) In those two years the figures were corrected based on the results in 2001 and 2002, and by HAAPANEN (1982).

Although the author knows the area very well each year some new breeding sites were found. Especially in 1999 and 2000 the sites were not fully covered However, most sites were checked each year. The sites surveyed every year ($n = 52$) covered 57 % of the egg clumps found in 2002 The size of the female spawning population and its annual variation were estimated based on the census figures from the sites surveyed each year 1999-2002.

The results of this study are compared with those obtained in part of this population in 1973-1977 (HAAPANEN, 1982).

RESULTS

SIZE OF THE FEMALE POPULATION

In 1999-2002 there were in average 786 ± 262 (mean and standard deviation) spawning females in the area The amount of the spawning frog females increased during the whole study period and especially from 1999 to 2000 (fig 3).

In the university farm the spawning female frog numbers were 81 ± 44 in 1999-2002, versus 58 ± 43 in 1973-1977 (HAAPANEN, 1982). The difference is not, however, significant ($P = 0.45$, t test).

SUB-AREAS

The spawning sites were found from about 0 to 12 5 m above sea level, but not in brackish waters.

During these four years 91 spawning sites were found on 18 green sub-areas (size 1-125 ha). They covered 70 % of all green areas.

Only one spawning site was found in the small house block in a dike connecting two woodlands. In six green areas no spawning sites were seen During the study period two sub-areas were lost as spawning area and one additional was found.

The numbers of female frogs varied greatly in these sub-areas. The average number of spawning females in 2002 was 63 ± 70/sub-area (range 3-248) The two sub-areas with 3 and 248 female frogs are located on both sides of a four-lane highway This big difference in frog numbers was observed in all four years.



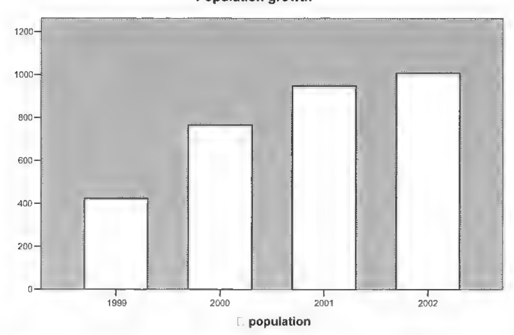


Fig. 3. – The growth of the spawning female common frog population in 1999-2002 in the study area in eastern Helsinki. The figures are the sums found in different sub areas.

There is a strong correlation between the number of egg clumps found in a sub-area and the number of breeding sites (Pearson $r = 0.87$). So the amount of spawning sites accounts for 76 % ($r^2 = 0.76$) of the total variation in the numbers of spawning females between the sub-areas. In contrast, there is only a very low correlation ($r = 0.29$) between the numbers of egg clumps and the size (ha) of the sub-areas. The size of the sub-area accounts for only 8 % of the numbers of the spawning females.

Population density in terrestrial habitats

No frogs were seen to spawn on rocky pine woodland areas although the latter are extensive in the study area and include some ponds. These pine woodlands account 30 % of those green areas where no breeding common frogs were found.

The highest population densities (9.1 and 7.0 female frogs/ha) were found in woodland parks and in woodlands, respectively. In semi-open areas and agricultural land, the densities were much lower (tab. 1). There are only two agricultural areas and these are grouped in tab. 1 with the semi-open areas. In agricultural land there were only 0.8 females/ha.

The spawning female frog density for the whole green area was 1.3/ha and in the whole study area it was 0.5/ha.

The spawning site density was so high (tab. 1) that the frogs could easily reach these sites in any type of habitat and vice versa.

SPAWNING SITES

The most common spawning site in the study area was the small dikes (tab. 2). One half of all egg clumps were laid in these dikes. All together the egg clumps were distributed within different spawning habitat types as these habitat types were used (tab. 3). The average number of egg clumps per site was almost the same (tab. 3).

Only 17 % of the spawning sites were used in every four years. In such areas the egg clump numbers were, however, high in each year (average 31 ± 27) and there is highly significant difference ($P < 0.001$, t test), with the average number of egg clumps (6 ± 6) in sites used only once or twice but not in following years. From 31 to 58 % of sites were empty. The lowest number of unused sites was found in 2002 when the population density was highest. The population increase did not cause the continuous increase of egg clump numbers per spawning site. The population increase was seen in the increase of the number of used spawning sites (tab. 4). In any spawning site the egg clump numbers varied considerably from year to year.

As the spawning sites are very small water bodies, they may easily become totally dry. A high proportion of all breeding sites became dry in early June 2002 when the metamorphosis of larvae had not yet taken place (tab. 5). There were no significant differences between the desiccation of dikes and ponds. All the brooks stayed watered. Some days after the inventory it rained so much that it is quite probable that the larvae in all remaining sites were able to metamorphose.

SPAWNING PATTERN AND START OF SPAWNING

The spawning started just after the average day temperature reached above 5°C. The spawning took 19 days in 2002. Most egg clumps were laid during the first days of spawning: half of egg clumps was laid already on the fifth day of spawning (fig. 2), and after ten days 80 % of all egg clumps has been laid, both in 2001 and 2002.

DISCUSSION

POPULATION SIZE AND DENSITY

The present common frog population is most probably the continuation of the former rural population. Taking into account the low survival rate in this species (50 % according to GIBBONS et al., 1984, 40 % according to LOMAN, 1984), the populations of the study area have lived several generations in the present situation of urbanisation.

The spawning sites of the study area are ideal for egg clump counting and the procedure developed gives quite exact figures of spawning female numbers.

Table 1. - Density of spawning females (female frogs/ha) and of spawning sites (sites/10 ha) in 2002 in different habitat types. Semi-open areas cover agricultural areas with woodlands, too. *x*, mean; *s*, standard deviation.

	Woodlands	Woodland parks	Semi-open areas
Population density ($x \pm s$)	7 ± 3.8	9.1 ± 1.6	2.1 ± 2.1
Number of areas	7	2	9
Spawning site density	6.8	9.6	1

Table 2. - Distribution of spawning sites ($n = 91$) between different habitat types and destruction and construction of sites in 1999-2002.

	Distribution %	Destruction *n*	Construction *n*
Dikes	51	3	0
Ponds	38	1	1
Brooks	7	0	0
Wider parts of brooks	4	0	1
Total	100	4	2

Table 3. - Distribution of spawning sites ($n = 63$) and of egg clumps ($n = 1007$) in 2002 within spawning habitat types, and mean numbers of egg clumps per site. *x*, mean; *s*, standard deviation.

	Dikes	Ponds	Brooks	Wider parts of brooks
Distribution of sites (%)	54	32	9	4
Distribution of egg clumps (%)	51	35	9	5
Number of egg clumps ($x \pm s$)	15 ± 15	18 ± 19	14 ± 21	17 ± 11

The frog population density of the study area is much lower than that (50-530 adults/ha) found by Loman (1984) in southern Sweden or that (64-80 adults/ha) found by Pasanen et al. (1993) in eastern Finland. Taking into account that, in these Finnish data, there were only 20 % females, the density figures in woodlands and woodland parks were of the same order of magnitude. The biased sex ratio in northern conditions may be caused by the slower development of the females (Loman, 1976; Gibbons et al., 1984).

Table 4. – Spawning in the 52 sites in 1999-2000, mean and median of egg clump numbers per site x, mean, s, standard deviation

	1999	2000	2001	2002
Percentage used as a spawning site (%)	44	42	50	69
Number of egg clumps/site ($x \pm s$)	11 ± 11	20 ± 24	21 ± 26	16 ± 20
Number of egg clumps/site (median)	26	43	43	39

Table 5. Results of the spawning site inventory on 5-10 June 2002. The figures show the sites which still were watered. Total number of sites surveyed: n = 58

	Dikes n (%)	Ponds n (%)	Brooks n (%)	Total n (%)
Watered sites	15 (52)	14 (70)	9 (100)	38 (52)
Egg clumps in watered sites	274 (57)	174 (50)	44 (100)	579 (60)

The results show that the common frog has for generations inhabited areas which seem to be quite fragmented and isolated, though frogs disappeared from one sub-area because the spawning sites were filled and the dikes were canalised.

SEPPÄ & LAURILA (1999) estimated that, in the conditions of the Baltic Sea small islands, 32 or more breeding females per island would result in an effective population. In my study area, 35 % of the populations in sub-areas were below this limit.

VOS & CHARDON (1998) found that the most decisive factor on the occurrence of the moor frog was the quality of habitat, not the degree of isolation. The data of this study show that small populations can survive at least several decades even close to highways in spite of the traffic mortality and the isolation.

Anuran population sizes vary because of variation in the size of annual cohorts (RYSER, 1986). This is quite evident in the case of small populations which are dependent on a small amount of spawning sites (see e.g. HAAPANEN, 1982). Here there were 91 different spawning sites available. This clearly levelled the annual population variations. Also in the present data the amount of spawning females in any spawning site varied considerably from year to year.

CONSERVATION REMARKS

In the study area the destruction of the habitat has not been a big problem during the study period (tab. 2). On the other hand some new sites were constructed. All the wider parts of brooks are a result of the restoration of a former canalized brook. The future succession of the vegetation will probably enhance these sites further.

The slow filling up of shallow dikes and ponds is a natural phenomenon which can destroy a great part of spawning sites in coming years. The city will be informed on the importance of the small water bodies as a frog habitat. The general plan provides certain protection of the summer habitat. Still the fragmentation of the population and especially the possible habitat loss make the future of the populations uncertain.

Limiting factors

This study allows to discuss whether the spawning habitat can be the density dependent factor limiting the common frog populations in these circumstances.

The summer range of these frogs can be measured as it is isolated from the surrounding by buildings and wide traffic lines. The frogs can easily reach the whole available terrestrial habitat as the distance to the spawning sites is not more than 500 m (see also tab. 1).

It was observed that the amount of spawning sites accounted for 76 % of the total variation in the population density. The size of the terrestrial habitat was only of secondary importance. It was also found that the amount of egg clumps per spawning site did not increase with the increase of the total number of egg clumps. Instead, with the increasing population, the number of spawning sites increased. The frogs apparently started to use the sites of secondary quality. So the number of spawning sites will be the ultimate limiting factor in situations when other factors, e.g. climatic conditions, have not caused the local decline of the population.

Résumé

L'étude porte sur la population de grenouilles rousses de la banlieue est de Helsinki, capitale de la Finlande (60°12'N, 25°08'E). La zone étudiée en 1999-2002 couvre 1 590 hectares, soit 8,5 % de la superficie totale de la ville. Elle a été urbanisée surtout dans les années soixante et soixante-dix, et sa population de grenouilles provient sans doute des grenouilles qui y vivaient avant cette période. Les grenouilles occupent les espaces verts de la zone. Ceux-ci couvrent un quart de la zone et se divisent en cinq catégories: (1) forêts de feuillus, (2) parcs boisés, (3) parcs à moitié ouverts avec pelouses entretenues, (4) terres arables; et (5) bois de pins sur terrain rocheux. Dans la catégorie 5 il n'y avait pas de grenouilles. Les espaces verts des quatre premières catégories sont divisés par des maisons, des rues et une route à quatre voies en 24 secteurs, dont 18 avaient des grenouilles au moins pendant une des années d'étude. Les masses d'œufs dans ces 18 secteurs ont été comptées dix jours après le début du frai ou un peu plus tard. Le résultat obtenu a été corrigé par le nombre de masses dans le secteur où l'on a pu suivre le frai jour par jour (fig. 1). Les frayères étaient des petits fossés, étangs, ruisseaux ou parties stagnantes des cours d'eau (tab. 2). La taille moyenne annuelle de la population de grenouilles en frai a été estimée à 786 (± 262) femelles dans la zone entière, et le nombre d'animaux a augmenté d'année en année (fig. 2). Dans les 18 secteurs où il y avait des grenouilles, leur densité moyenne était de 1,3 femelles par hectare. Celle-ci était la plus grande dans les habitats boisés (tab. 1). Une corrélation significative (r = 0,87) a été constatée entre le nombre de femelles en frai par secteur et le nombre de frayères. La taille du secteur n'est corrélée qu'avec 7 du nombre total des femelles en frai. L'accroissement du nombre de frayères utilisées est allé de pair avec l'accroissement de la population de grenouilles, mais le nombre de masses d'œufs par frayère a augmenté moins vite. La conclusion est que c'est le nombre de frayères qui limite la taille de la population des grenouilles. Quand la population croît, une partie des femelles est obligée de choisir des frayères suboptimales.

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A preliminary biotelemetric study of a feral invasive *Xenopus laevis* population in France

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The invasive African clawed frog (*Xenopus laevis*) is currently spreading over a large area in western France. In order to investigate the population expansion processes we studied the feasibility of implanted transmitters use. Seven frogs were radiotracked during the winter period. Even in this cold period of the year, individual movements were observed in the natural water network, and also in the flooded terrestrial surrounding area. These areas play a key role in the invasive process. During the study, freezing and predation by the polecat (*Mustela putorius*) seemed to be the major adult mortality factors.

INTRODUCTION

Introduction of non-native organisms into the wild for economic, sport, aesthetic reasons, or accidentally, are very common processes occurring at a growing rate since the last century. If in many cases non-native organisms may be harmless in their new environment, in other cases they prone to escape human control and could become invasive (WILLIAMSON, 1996). Like many animal groups, amphibians have also been the subjects of the invasive process. The African clawed frog, *Xenopus laevis*, is one of the known invasive frog species, currently established in many non-native area, principally in California, Arizona and north Mexico since the sixties (CRAYON, in press), and in Chile and south Wales since the seventies (MEASEY & TINSLEY, 1998; LOBOS et al., 1999, LOBOS & MEASEY, 2002). Many other more or less isolated populations have also been noticed, including on Ascension Island in the south Atlantic Ocean since 1944 (TINSLEY & MCCOID, 1996, CRAYON, in press). Quite recently feral African clawed frogs have been discovered in western central France (FOUQUET, 2001) and are suspected to have become established since the eighties. It may be the largest known European population since its known range was about more than 100 square kilometers in 2003, which is likely to be largely underestimated and quickly increasing (FOUQUET & MEASEY, 2006).

According to climatic conditions, French feral *X. laevis* suffer almost the same conditions as in south Wales, which have been described as ill-suited to this southern African species

(MEASEY & TINSLEY, 1998). The south Wales populations have been intensively studied regarding their demographic parameters and feeding habits (MEASEY & TINSLEY, 1998; MEASEY, 1998, 2001), and they seem to occur only within a limited area (MEASEY & TINSLEY, 1998). A skeletochronological investigation shows that successful recruitment infrequently occurs (MEASEY & TINSLEY, 1998, MEASEY, 2001), potentially limiting *X. laevis* spread. Therefore, the dispersal success of *X. laevis* in the French countryside calls for some explanation. Surprisingly, the African clawed frog, despite being a standard for developmental, physiological or molecular laboratory studies, remains poorly known regarding its population ecology, even in its native habitats (MEASEY, 2004). The goals of this study are (1) to test the use of implantable transmitters to track clawed frogs in the wild, and then (2) to observe frogs' movement and winter mortality during cold wet season in the area inhabited in France.

MATERIAL AND METHODS

STUDY AREA

We chose one of the numerous colonized ponds of the current frog's distribution, according to the following criteria, permanent pond, resembling many other colonized ponds and surrounded by a maximum diversity of landscapes, not situated in the border of the occupied area, not holding a high density of African clawed frogs. The chosen pond was located near Vibreuil (46°59'N, 00°19'E), in the middle of an extensive pasture, surrounded by typical traditional hedges, including small groves, wooded hedges and ditches (fig. 1), and also ploughed fields. The pond, shaped with strong sloping banks except on one side, serves as watering place for some cattle. Its depth was about 200 cm maximum during the study. It was free of fish, contained very little vegetation, and during the study few other amphibian species were caught (*Triturus cristatus*, *T. helveticus*). The pond was supplied with water by small ditches collecting rainwater from the nearby pasture area, but also sometimes by overflow from the same continuous small ditches which are connected further up to a larger water network. The pond was connected to the water network only during the wet seasons, i.e., probably only a few months each year.

SAMPLING OF CLAWED FROGS AND TELEMETRIC PROCEEDINGS

African clawed frogs were caught using funnel traps baited with pieces of meat (FOUQUET & MEASEY, 2005) from November 2002 to February 2003. Traps were set for one or two consecutive nights in the water. Then frogs were brought to the lab for transmitters implantation. They were sexed, weighed and measured with a calliper to the nearest millimeter. According to the implantation method described by EGGERT (2002), frogs were anaesthetized and transmitters (Sirtrack, Single Stage Transmitters) were placed through a small incision in the body cavity. The abdominal muscles and skin layers were then sutured together in two separate layers. The animals were kept for a few days in aquarium to verify full recovery before releasing in the exact place of capture. Animals were located about once a week, sometimes

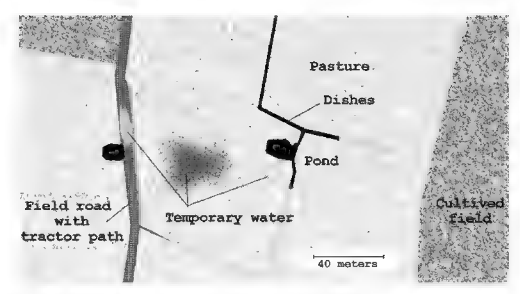


Fig 1 – Situation plan of the studied area of feral clawed frogs in France.

less during very cold weather conditions. They were located with at least half a meter accuracy. When death of a frog was suspected in the water, we tried to catch it with a landing net.

Results

Seven frogs (4 males and 3 females) were caught in the pond and then tracked during winter (tab. 1).

Most of the frogs' movements were limited to the pond, but sometimes frogs went out of it. Thus 19.6 % of the frog locations were situated in surrounding dishes and 21.6 % in temporary puddles. Only one individual (female 696) did not leave the pond but after 8 days the transmitter was found alone and damaged some meters out of the pond in the pasture. In the same way a male (male 555) was predated after a two weeks trip in the small ditches upstream from the pond. In both cases we assume that the western polecat (*Mustela putorius*) was the predator (polecat faeces were found very close to the still working transmitters). Two males were tracked until transmitter signals were lost for unknown reasons, but in both cases polecat action is suspected. One was lost just after releasing, while the other (male 1036, see fig. 2) was tracked for two months. Two dead individuals were found in the pond, close to its border, without any evident cause of death. One (male 059) had shown a constant movement activity (but mainly in the pond) during the 3 weeks of tracking, whereas the other (female 436) was found dead only one week after release. In both cases post-operative problems cannot be excluded, even if posterior autopsies have not revealed any apparent injuries, except a slight inflammation in the region of the incision.

Table 1 – Some data on radiotracked feral clawed frogs in France (November 2002-February 2003)

Sex/code	Size mm	Mass g	Date of capture	Last control	Cause of loss
Male 059	69	45 0	17 November	14 December	Death
Male 555	68	47 0	16 November	01 December	Predated
Male 696	71	42.7	09 February	20 February	Unknown
Male 1036	74	46.7	13 December	16 February	Unknown
Female 436	89	85.7	23 November	14 December	Death
Female 398	94	99 1	23 November	16 February	Dead frozen
Female 696	99	114 1	23 November	14 December	Predated

The first two weeks of December were cold (but without freezing), whereas the two last were milder (a temperature up to 10°C during the day was observed). January was very cold, with most of the night temperatures below 0°C, like in mid-February. Soil and water became colder during January, freezing during the first week of February At that moment, all wetland habitats were covered with 10 centimeters of ice. One individual (female 398) which moved about 80 meters from the pond (fig 2), moved overland through pasture, crossing a wooded hedge then was located in a puddle 20 centimeters deep. It died in early February by freezing.

DISCUSSION

IMPLANTATION PROCEDURE

As laboratory kept frogs often perform an overhead kicking movement with their clawed feet, it was necessary to sew up the suture using a large amount of skin Moreover it was not possible to keep clawed frogs for a long time in dry conditions, so that healing was considerably longer than in terrestrial amphibians (pers. obs.) Stitches of one female break just after sewing up and therefore we sewed them again with a larger suture, with a larger recovering of the two facing skin parts We suggest using absorbable gut for the muscle layer and nylon suture for the skin closure Also broad-spectrum antibiotics to prevent infections in the wild could be tested Likewise avoiding cold water temperatures during healing process may increase healing rate (COLBERG et al., 1997).

CLAWED FROGS MOVEMENTS

In spite of the rather cold weather conditions during the course of our study, clawed frogs' movements were not limited to the pond Trips in the connected small dishes, with lower

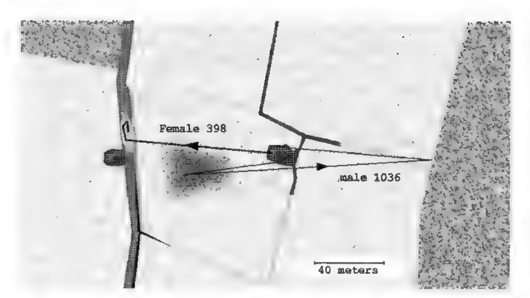


Fig. 2. – Example of clawed frogs movement in the study site during the tracking period (see text). The other tracked frogs did not move further than these.

water level (maximum about 40 cm), were observed, as well as overland movements. Therefore, during winter, clawed frogs could be found not only together in ponds or rivers, but also alone or in small numbers in small temporary puddles unconnected with permanent or temporary streams. The use of such temporary water places, that are numerous in this agricultural region, should clearly be considered in any planned eradication program. Moreover, clawed frogs are able to move even in quite cold weather conditions. By marking individuals during several years in the UK, Measey & Tinsley (1998) observed that less than 36 % of the frogs were moving between capture sites, mainly over few hundred meters, with a maximum of two kilometers along a river valley. Overland movements could occur through woodland with dense undergrowth, over metalled roads and also across rivers. In our study, leaving the pond was associated with high risk of mortality by contact with predators or by freezing in a temporary water surface (also several young *X. laevis* have been found dead in a shallow pond after a cold period; pers. obs.). Nevertheless, the relationship between animals with implantable transmitters and predation probability remains to be studied. Severe winters have been proposed as a major factor affecting clawed frog introduction success in European area (Frazer, 1964). Freezing or suffocation underneath ice layer have long been reported for European amphibians (e.g. *Rana temporaria* in De la Fontaine, 1881). It was obviously a cause of *X. laevis* mortality in France but clearly does not prevent its invasion.

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Laurenti revisited

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Josephus Nicolaus LAURENTI, 1768 · ***Specimen medicum, exhibens synopsin Reptilium emendatam cum experimentis circa venena et antidota Reptilium Austriacorum. Facsimile reprint with an English translation by Sergius L. Kuzmin*** Laurenti Verlag, Supplement der *Zeitschrift für Feldherpetologie*, **7**, 2005: 1-247. ISBN 3-933066-24-7

Reprinting old volumes in zoology has gained some popularity in recent times. In many cases, beautiful illustrations provided the main attraction of such an enterprise. So why should anybody be interested in what ADLER (1989) called an "unimposing little book" with just five black-and-white plates of illustrations?

Laurenti's treatise contains two parts, a systematic overview of the "Reptilia" (including amphibians) and a natural history of Austrian "reptiles", with some detailed descriptions and remarkable observations on natural history, including eighty-nine carefully detailed experiments on the venoms of a number of species. Viewed from a local patriotic viewpoint, this book opens the chronicle of herpetological research in Austria (which was a large empire at that time) (TIEDEMANN, 2001). Its main interest for a wider audience is based on the many genus and species names first proposed here, making it an important resource for taxonomy and nomenclature even today. Much of its scientific content, especially the toxicological work, is clearly outdated and will be read mainly for curiosity or historical interest. Nevertheless, I found — both in the descriptive and in the experimental sections — many statements, discussions and stories that stimulate reflections on the state and development of science then and now.

The book starts with two prefaces, by Burkhard Thiesmeier and Wolfgang Böhme, and an introduction by Sergius Kuzmin. Then Laurenti's treatise is presented, the facsimile on the right hand pages, with the English translation on the opposite pages, followed by the illustrations. Finally, the translator provides a few comments, a list of books of the authors mentioned by Laurenti, a list of valid scientific names for species mentioned by Laurenti, and references for main sources of information.

Sergius Kuzmin has undertaken the difficult task of translating the text from one foreign language into another. One can find flaws and minor mistakes in the translation if one looks for them, but by and large Kuzmin has succeeded remarkably well in providing a readable and correct English version of Laurenti's work. The treatment of geographic terms, both Latin and German ones, is slightly inconsistent as sometimes a modern spelling is given (e.g. "Wieden" for "Widen", "Dauphine" for "Delphinatu") whereas in other cases an outdated spelling is directly taken into the English text (e.g. "Nusdorff" or "Smolandia"). The location "In alpe Etscher" or "In Etschero monte" (type locality for *Triturus alpestris*) is repeatedly rendered as "Escher mountain" though the name of this mountain is Ötscher (ROČEK et al., 2003, gave the incorrect spelling Otscher).

The publisher is to be commended for making available this important classical work to a wider audience.

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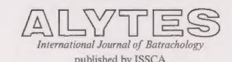
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